CLINICAL STUDY PROTOCOL

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A Phase I/II Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) rh10-Mediated Gene Transfer of Human Factor IX in Adults With Moderate/Severe to Severe Hemophilia B

101HEMB01

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Amendment 2: 29 July 2015
Amendment 3: 03 September 2015

CONFIDENTIAL

The study will be conducted according to the International Conference on Harmonisation harmonised tripartite guideline E6(R1): Good Clinical Practice.

Protocol Approval

Study Title	A Phase I/II Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) rh10-Mediated Gene Transfer of Human Factor IX in Adults With Moderate/Severe to Severe Hemophilia B
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Protocol accepted and approved by:

Sponsor Signatory

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Protocol Amendment History and Summary of Changes

Version	Date	Summary of Changes
Version 01	10 April 2015	Original Protocol
Version 02	10 June 2015	Overview of significant changes includes the following: • The protocol was originally designed to seamlessly move from a Phase 1/2 (Part 1) into a larger registration study (Part 2). Part 2 has been removed from this study and will be a separate study. The text has been revised throughout the protocol to reflect this change. • Assessment of the subject's quality of life (QoL) following treatment with DTX101 was originally planned for Part 2 of the study. With the removal of Part 2, the QoL questionnaires have been included as a secondary endpoint in this dose-finding study. • The frequency of clinical chemistry samples to monitor liver function tests (LFTs) has been increased to occur approximately every 4 days for the first 12 weeks after DTX101 administration to ensure that any elevation in LFTs, potentially as a result of autoimmune hepatitis, are discovered quickly and adequately managed. • The sampling scheme monitoring for viral shedding has been increased from 3 weeks to 12 weeks. • The frequency of site visits over the first 12 weeks has been reduced from weekly to bi-weekly (Week 2 through Week 12). • In order to accommodate the increased frequency of clinical chemistry sampling, subjects will be seen at home by clinically trained and qualified personnel approximately every 4 days (unless a study visit is

		scheduled) to minimize the frequency of study visits. • Elevated liver function test results associated with potential autoimmune hepatitis and infusion site reactions are no longer designated as adverse events (AEs) of special interest. They will continue to be recorded as AEs/serious adverse events (SAEs) in the clinical database.
Version 03	29 July 2015	Overview of significant changes include the following: • The no observed adverse event level (NOAEL) used to develop the rationale for the starting dose of DTX101 was originally determined from a non-Good Laboratory Practice (GLP) nonclinical toxicology study and reported as 1.35×10^{13} genome copies (GC)/kg. A GLP-compliant nonclinical toxicology study has recently been completed and the NOAEL has changed to 5.0×10^{12} GC/mL. The dosing rationale and safety margins for each of the proposed doses of DTX101 have been revised to reflect this change. • The development of AAVrh10 binding antibodies has been added as an exploratory endpoint. • The optimal biological dose has been updated to be the dose that achieves or is closest to achieving the target peak Factor IX activity of ≥20% of normal. • Amendment 1 of the protocol added home visits to accommodate the increased frequency of clinical chemistry sampling. The language has been revised to allow subjects to either visit the clinic or have samples taken at home by clinically trained and qualified personnel. • The safety stopping criteria has been updated to clarify that the study will be suspended so that the results can be reviewed and any risks to subjects can be mitigated.

Version 04	03 September 2015	Overview of significant changes include the following: • Exclusion Criterion 2 was revised so that subjects with alanine aminotransferase and aspartate aminotransferase elevations >2.0 × the upper limit of normal will not be eligible for the study. This has been reduced from >3.0 × the upper limit of normal. • Language has been added to reflect that an interim analysis will be performed once the last subject enrolled in the study has completed Week 6.

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Protocol Synopsis

Protocol Number:	101HEMB01
Title:	A Phase I/II Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) rh10-Mediated Gene Transfer of Human Factor IX in Adults With Moderate/Severe to Severe Hemophilia B
Sponsor:	Dimension Therapeutics, Inc 840 Memorial Drive Cambridge, MA 02139
Study Phase:	I/II
Study Sites:	Up to 14 global study sites.
Sample Size:	The study is anticipated to enroll approximately 12 to 18 subjects.
Indication:	Moderate/severe to severe hemophilia B
Primary Objectives:	• To determine the safety of single ascending intravenous (IV) doses of DTX101 in adults with moderate/severe to severe hemophilia B. • To establish a dose of DTX101 that achieves a peak plasma level of vector-derived factor IX (FIX) at 6 weeks after IV administration to allow further clinical development.
Secondary Objectives:	• To assess the impact of DTX101 on the number of bleeding episodes requiring recombinant FIX infusion during the study. • To evaluate the kinetics, duration, and magnitude of plasma FIX activity, by dose, after IV administration of DTX101 in adults with hemophilia B. • To assess the impact of DTX101 on the frequency of FIX replacement therapy during the study. • To describe the immune response to the FIX transgene after IV administration of DTX101. • To assess the impact of DTX101 on the subject's quality of life.

Exploratory Objective:	• To describe the immune response to adeno-associated virus serotype rh10 (AAVrh10) capsid proteins after IV administration of DTX101.
Primary Endpoints:	• The incidence of adverse events (AEs), treatment-emergent adverse events (TEAEs), and serious adverse events (SAEs), will be summarized for each dosing cohort by severity and relationship to study product. • The change from baseline in FIX activity at Week 6 as determined by the activated partial thromboplastin time (aPTT) clot-based assay.
Secondary Endpoints:	• The annualized bleeding rate (ABR) will be calculated for all subjects through Week 52 (±7 days). • The time course of FIX activity, as determined by aPTT, will be summarized by time point and dose level of DTX101. • The annualized and average weekly use of FIX replacement therapy will be calculated for all subjects through Week 52 (±7 days). • The development of neutralizing antibodies to FIX (FIX inhibitor), as determined by a Bethesda assay, will be summarized by time point and dose level of DTX101. • The development of a cell-mediated immune response to FIX, as determined by enzyme-linked immunospot (ELISPOT) assay, will be summarized by time point and dose level of DTX101. • Responses to the EuroQol 5D 5 level (EQ-5D-5L™) and Haemophilia-Specific Quality of Life (Haem-A-QoL) questionnaires will be summarized.
Exploratory Endpoints:	• The development of neutralizing antibodies to AAVrh10, as determined by enzyme-linked immunosorbent assay (ELISA), will be summarized by time point and dose level of DTX101. • The development of a cell-mediated immune response to AAVrh10, as determined by ELISPOT assay, will be summarized by time point and dose level of DTX101. • The development of anti-AAVrh10 binding antibodies, as determined by ELISA, will be summarized by time point and dose level of DTX101.

Subject Population:	**Inclusion Criteria** Each subject must meet all of the following criteria at screening to be enrolled in this study: 1. Male ≥18 years of age. 2. Moderate/severe or severe hemophilia B (baseline FIX activity ≤2% of normal or documented history of FIX activity ≤2%). 3. At least 3 bleeding episodes per year that require on-demand treatment with FIX OR are treated with a prophylactic regimen of FIX. 4. At least 100 days exposure history to FIX. 5. No documented history of inhibitors (neutralizing antibodies) to exogenous FIX. 6. No known allergic reaction to exogenous FIX or any component of DTX101. 7. Willing to stop prophylactic treatment with recombinant FIX at specified time points during the study. 8. Willing and able to provide written informed consent. 9. Willing and able to comply with study procedures and requirements. 10. Willing to use effective contraception at the time of administration of DTX101 and for 3 months following administration of DTX101. Appropriate contraceptive methods include a condom with spermicide. Abstinence, defined as sexual inactivity, is an acceptable form of birth control; however, appropriate contraception must be used if the subject becomes sexually active. **Exclusion Criteria** Subjects meeting any of the following criteria at screening will be excluded from the study: 1. History of liver disease as evidenced by any of the following: portal hypertension, ascites, splenomegaly, esophageal varices, hepatic encephalopathy, or a liver biopsy with evidence of stage 3 fibrosis. 2. Significant hepatic inflammation or cirrhosis as evidenced by any of the following: aspartate aminotransferase (AST) or alanine aminotransferase

	(ALT) >2.0 × upper limit of normal (ULN), total bilirubin >1.5 × ULN, alkaline phosphatase (ALP) >2.5 × ULN, platelet count <75,000 cells/μL, or prothrombin time (PT) or international normalized ratio (INR) >1.5 × ULN. 3. Evidence of active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, documented by current use of antiviral therapy for HBV or HCV or by hepatitis B surface antigen (HBsAg) or HCV RNA positivity. NOTE: Two negative viral assays by polymerase chain reaction (PCR), collected at least 6 months apart, will be required to be considered negative for HCV. Subjects can be rescreened once if they have one negative sample and must wait to have the second sample collected within the following 6 months. 4. History of human immunodeficiency virus (HIV) infection AND any of the following: CD4+ cell count <350 cells/mm^3, change in antiretroviral therapy regimen within 6 months prior to Day 0, or plasma viral load >200 copies/mL, on 2 separate occasions, as measured by PCR. 5. Anti-AAVrh10 neutralizing antibody titer >1:5. 6. Participation (current or previous) in another gene therapy study. 7. Participation in another investigational medicine study within 3 months before screening. 8. History of a malignancy for which the subject has received treatment in the past 2 years except for prostate cancer treated with watchful waiting, or surgically removed non-melanoma skin cancer. 9. Has any other significant medical condition that the investigator feels would be a risk to the subject or would impede the study.
Study Design:	This is a Phase I/II, open-label, single-arm, multicenter, dose-finding safety study to determine the safety, tolerability, and efficacy of DTX101 in adult males with moderate/severe to severe hemophilia B. The primary objectives of the study are to determine the safety of single ascending IV doses of DTX101 and to identify the optimal biological dose (OBD) of DTX101

	that either achieves or is the closest to achieving the target peak FIX activity of ≥20% of normal. Eligible subjects will receive a single IV infusion of DTX101. Subjects will be dosed sequentially in cohorts with a minimum of 3 subjects each. Dose escalation will be conducted according to a model that uses safety and efficacy data to predict the OBD. The recommendation to proceed to the next dose cohort will be made by the Data Safety Monitoring Committee after evaluation of the safety data for all subjects in a dosing cohort after they have completed Week 6. Subjects will be followed for 52 weeks after dosing. After completion of this study, subjects will be offered enrollment in an extension study to evaluate the long-term safety and effect of DTX101 on clinical outcome measures.
Study Methodology:	**Screening Period** After a subject has provided written informed consent, and within 30 days before DTX101 infusion (Day 0), the investigator or other qualified study personnel will determine if the subject is eligible for the study. This will be accomplished by reviewing the inclusion and exclusion criteria and completing all of the screening assessments. If the subject does not have a documented history of FIX activity, a blood sample will be needed to confirm the severity of hemophilia B for inclusion after washout of recombinant FIX. For subjects taking long acting recombinant FIX prophylactically to prevent bleeding episodes, the washout period is to start at approximately Day -29 and last for 21 days (approximately Day –8). For subjects taking traditional recombinant FIX prophylactically to prevent bleeding episodes, the washout period is to start at approximately Day -29 and last for 7 days (approximately Day -22). The screening assessments may be performed on more than 1 day, provided that all of the assessments are completed and the results are available within the 30-day screening window and prior to Day 0. Screening Assessments: Demographic data; medical and hemophilia history; prior medications, therapies, and procedures; vital sign measurements; height and weight will be recorded. A 12-lead electrocardiogram (ECG) and a complete physical

	examination will be performed. Samples will be collected for FIX and human leukocyte antigen genotyping; HIV, HBV, and HCV status; FIX inhibitor determination; AAVrh10 binding antibody immunoglobulin G (IgG) assay; AAVrh10 neutralizing antibody testing; FIX activity; clinical laboratory assessments (hematology, coagulation, clinical chemistry, and urinalysis); and record spontaneous bleeding episodes and recombinant FIX use. **Day 0 – DTX101 Infusion** Subjects will be admitted for DTX101 administration and for continuous safety monitoring. Subjects will be discharged 24 hours after completion of the infusion. Prior to dosing, blood samples will be collected for FIX inhibitor determination, AAVrh10 binding antibody IgG assay, AAVrh10 neutralizing antibody testing, FIX activity, cell-mediated immune response to AAVrh10 and FIX, and clinical laboratory assessments (hematology, coagulation, and clinical chemistry). Saliva, urine, and stool samples will be collected for assessment of viral shedding. An ECG (in triplicate) and a targeted physical examination will be performed. Vital sign measurements and weight will be recorded. The use of concomitant medications, spontaneous bleeding episodes and recombinant FIX use will be recorded. Subjects will be provided a paper or electronic diary (eDiary). The subject will complete the EQ-5D-5L and the Haem-A-QoL questionnaires. After dosing, a sample will be collected for clinical laboratory assessments (hematology and clinical chemistry) at 0.5, 4, and 8 hours after the start of infusion. Vital sign measurements will be recorded at 5 minutes after the start of infusion, and at 0.5, 1, 2, 4, 6, 8, and 22 hours after the start of infusion. A single 12-lead ECG will be performed at 1 hour after the start of infusion. **At All Visits (Day 1 Through Week 52)** Subjects will be asked to visit the study site approximately every 4 days through Week 12. Following the Week 12 visit, subjects will visit the study site once every 4 weeks through Week 52 or

	their early withdrawal from the study. At each visit, samples will be collected for FIX activity and clinical laboratory assessments (hematology, coagulation, and clinical chemistry). Vital sign measurements will be recorded. Adverse events and SAEs will be monitored; concomitant medications, therapies, and procedures will be recorded; and the paper or eDiary will be reviewed with the subject. **Clinic or Home Visits** Subjects will be asked to provide clinical laboratory samples approximately every 4 days through Week 12 of the study. If subjects cannot visit the study site in person, subjects can be visited by clinically trained and qualified personnel approximately every 4 days at their home through Week 12, the exception being those weeks when a site visit is scheduled, to collect a clinical laboratory sample to monitor liver function tests (LFTs). Saliva, urine, and stool samples will also be collected for assessment of viral shedding on Days 8, 20, 36, 48, 64, and 76. These samples will continue to be collected until 3 consecutive negative results are obtained from each sample matrix. **Weeks 2, 4, 6, 8, 10, and 12** Saliva, urine, and stool samples will be collected for assessment of viral shedding. These will continue to be collected until 3 consecutive negative results are obtained from each sample matrix. Subjects will be asked to provide a sample for urinalysis (Weeks 2, 4, 6, 8, and 12). In order to accurately assess vector-derived FIX activity as a result of DTX101 infusion, subjects will stop taking FIX prophylactically prior to the assessment of peak FIX activity at Week 6 and transition to an on-demand regimen for the remainder of the study. Subjects prophylactically using traditional recombinant FIX will be asked to begin the washout period starting at Week 5. Due to the longer half-life, subjects prophylactically taking long-acting recombinant FIX (eg, Alprolix) will be asked to begin the washout period starting at Week 3.

	Weeks 6, 8, 16, 32, 40, and 48 Samples will be collected for FIX inhibitor determination, coagulation panel, AAVrh10 binding antibody IgG assay, AAVrh10 neutralizing antibody testing, and cell-mediated immune response to AAVrh10 and FIX. If, at any time during the study, a subject experiences bleeding episodes frequent enough to consider prophylactic treatment, the investigator will discuss treatment options with the medical monitor. If a subject resumes prophylactic FIX replacement therapy, as rescue treatment after Week 6, additional washout periods prior to Week 24 and Week 52 will also be performed. Subjects prophylactically using traditional recombinant FIX will be asked to begin the washout period starting at Week 23 and Week 51. Due to the longer half-life, subjects taking long-acting recombinant FIX (eg, Alprolix) will be asked to begin the washout period starting at Week 21 and Week 49. **Weeks 6, 12, 24, 36, and 48** The subject will complete the EQ-5D-5L and the Haem-A-QoL questionnaires (Weeks 24, 36, and 48 only). A targeted physical examination will be performed. **Week 52/Early Withdrawal** Samples will be collected for FIX inhibitor determination, coagulation panel, AAVrh10 binding antibody IgG assay, AAVrh10 neutralizing antibody testing, and cell-mediated immune response to AAVrh10 and FIX. The subject will complete the EQ-5D-5L and the Haem-A-QoL questionnaires. A complete physical examination will be performed. A 12-lead ECG will be performed.
Steroid Use:	Steroid treatment for potential vector-induced immune hepatitis will be allowed if there is a >1.5-fold increase from baseline (predose, Day 0) in the subject's ALT that is considered related to treatment with DTX101 by the investigator. Within 24 hours of learning of the elevation, the site will repeat the test at their local laboratory and, if still elevated, will initiate steroid treatment following the treatment algorithm outlined in this protocol.

Safety Stopping Criteria:	Enrollment will be suspended and the study will be discussed with regulators if, at any time during the study, any of the following occur: • Death of a subject, at any time, that is considered related to DTX101 by the investigator, • Fivefold increase from baseline (predose, Day 0) ALT within 8 weeks after vector infusion that is considered related to DTX101 by the investigator, • Grade 2 toxicity develops that persists for more than 7 days and that is considered related to DTX101 by the investigator, • At least Grade 3 toxicity develops, including an infusion-related reaction ≥Grade 3, that is considered related to DTX101 by the investigator, or • Occurrence of a malignancy, at any point after administration of vector, that is considered related to DTX101 by the investigator. Abnormal clinically significant laboratory values (hematology, coagulation panel, and clinical chemistry) as assessed by the investigator will be considered AEs. Laboratory values that meet any of the above criteria will result in the suspension of the study. Any event that meets the above criteria will be reported immediately and captured as an AE/SAE in the electronic case report form (eCRF), as appropriate. Enrollment and dosing will be temporarily suspended until the situation can be assessed and risks to subjects mitigated. If study enrollment is suspended, all subjects who have been enrolled will remain in the study and will continue to be monitored through their completion or withdrawal from the study. All AEs will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03.
Efficacy Assessments:	Efficacy will be assessed based on the change from baseline in FIX activity, the number of bleeding episodes, and the dosing requirements for FIX replacement therapy. **Factor IX Activity** The change from baseline in FIX activity at Week 6 and at

	additional time points throughout the study will be assessed. Factor IX activity will be determined using an aPTT clot-based assay. **Bleeding Episodes** Bleeding episodes will be recorded in a paper or eDiary and will include: the start/stop date, the location of the bleed (joint, muscle, skin/mucosa, or internal), and the type of bleed (spontaneous or traumatic). The start date of a bleeding episode is defined as the first onset of symptoms of a bleed. The stop date of a bleeding episode is defined as 72 hours after the last on-demand treatment for a bleed, during which no additional bleeding episodes were noted in that location. If a bleeding event occurs in the same location within 72 hours of on-demand therapy, it will be considered part of the same episode. The site will review the diary with the subject at each clinic visit and record bleeding episodes on the appropriate page of the eCRF. Any event considered an AE should be recorded on the appropriate page of the eCRF. The annualized number of bleeds per subject, per location, and by type of bleed will be calculated for all subjects through Week 52 (±7 days). **Factor IX Replacement Therapy** The use of on-demand FIX replacement therapy will be recorded in a paper or eDiary and will include: the date(s) and dose (IU/kg) administered. The site will review the diary with the subject at each clinic visit and record the use of FIX replacement therapy on the appropriate page of the eCRF. The annualized and average weekly use of FIX replacement therapy will be calculated for all subjects through Week 52 (±7 days).
Safety Assessments:	Safety will be assessed based on AEs, SAEs, complete and targeted physical examination findings (including weight), vital sign measurements, ECG results, clinical laboratory assessments (hematology, coagulation panel, clinical chemistry, and urinalysis), viral shedding, measurement of neutralizing antibody titer to AAVrh10, measurement of AAVrh10 binding antibodies, measurement of FIX inhibitor, and assessment of any cell-mediated immune responses to AAVrh10 and FIX.

Estimated Study Duration:	The duration of the study is defined for each subject as the date signed written informed consent is provided through the visit at Week 52. Subjects will be in the study for approximately 56 weeks (including the screening period).
Study Product, Dose, and Route of Administration:	DTX101 will be administered as a single peripheral IV infusion. The following dose candidates will be assessed using a continual reassessment method (CRM) method to determine OBD: • Dose 1: 1.6×10^{12} GC/kg • Dose 2: 3.0×10^{12} GC/kg • Dose 3: 5.0×10^{12} GC/kg • Dose 4: 1.0×10^{13} GC/kg
Statistical Methods:	**Determination of the Optimal Biological Dose** A CRM will be used for dose-finding in this study. A 2-parameter logistic regression model will be used to predict both the probability of experiencing a dose-limiting toxicity (DLT) and the probability of achieving the target peak FIX activity (≥20% of normal) within the first 6 weeks after DTX101 infusion. A noninformative prior, in the form of a product of beta and exponential density functions, will be used. The maximum tolerated dose (MTD) is the dose when the posterior probability of the dosing cohort experiencing a DLT, based on the NCI CTCAE v4.03, is ≥25%. For this study, a DLT is defined as any AE/SAE ≥Grade 3 that is considered to be related to study product by the investigator. The MTD can also be the OBD if target FIX activity is achieved. A dose will be considered the minimal effective dose (MED) if the predicted probability of achieving the target peak FIX activity (≥20% of normal) reaches 90%. At each dose, a Euclidian distance between the posterior probabilities of safety and efficacy signals will be minimized to identify the dose for the next cohort. Subjects will be dosed sequentially in cohorts of a minimum of 3 subjects each. Subjects in the first cohort (Cohort 1) will be assigned to Dose 1. Subjects will be assigned to subsequent cohorts depending on the safety and FIX activity of the preceding doses. The potential dosing scheme allows for, at most, bypassing one dose level (eg, Dose 1 to Dose 3) during dose-finding. Similarly, the potential dosing scheme allows for

	de-escalation if the MTD is reached as well as cohort expansion to confirm a dose is the OBD. The protocol will also allow for adding an intermediate dose, relative to the candidate doses, if the CRM predicts it could be the OBD. Dose escalation will stop if at least one of the following conditions are met: 1) The MTD is reached; 2) The MED is found; 3) If a dose is sufficiently close to either the MTD or MED (within 5% credible interval); 4) If the posterior probability of a dose being either the MTD or the MED is over a threshold (10% and 20%, respectively); 5) If all of the dosing cohorts have been enrolled. **Interim Analysis** An interim analysis will be conducted when the last subject dosed completes Week 6 of the study. The purpose of this interim analysis is to determine FIX activity in all cohorts to determine the OBD of DTX101 and provide an initial safety and efficacy assessment of DTX101. **Safety Analyses** All statistical analyses of safety outcomes will be descriptive. The incidence of AEs and TEAEs will be summarized for each dosing cohort by severity and relationship to study product. Serious AEs will be presented for each dosing cohort by relationship to study product. Summary tables will present incidence estimates and individual event rates by system organ class as well as within each system organ class. Subjects experiencing an event more than once with varying severity will be counted only once, in the maximum severity within each system organ class and preferred term. For incidence of relationship to study product, subjects will be counted only once, in the category of the strongest relationship to study product within each system organ class and preferred term.

List of Abbreviations

Abbreviation	Definition
AAV	adeno-associated virus
AAV2	adeno-associated virus serotype 2
AAV8	adeno-associated virus serotype 8
AAVrh10	adeno-associated virus serotype rh10
ABR	annualized bleeding rate
ADL	activities of daily living
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
CFR	Code of Federal Regulations
CRA	clinical research associate
CRM	continual reassessment method
CTCAE	Common Terminology Criteria for Adverse Events
CTLs	cytotoxic T lymphocytes
DLT	dose-limiting toxicity
DSMC	Data Safety Monitoring Committee
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
eDiary	electronic diary
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
EQ-5D-5L	EuroQol 5D 5 level
EQ-VAS	EuroQol visual analogue scale
FDA	US Food and Drug Administration
FIX	factor IX
GC	genome copies
GCP	Good Clinical Practice

Abbreviation	Definition
GLP	Good Laboratory Practice
Haem-A-QoL	Haemophilia-Specific Quality of Life questionnaire
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IB	Investigator's Brochure
IBC	institutional biosafety committee
ICF	informed consent form
ICH	International Conference on Harmonisation
IEC	independent ethics committee
INR	international normalized ratio
IRB	institutional review board
IV	intravenous
LFT	liver function test
MED	minimal effective dose
MedDRA	Medical Dictionary for Regulatory Activities
MTD	maximum tolerated dose
NCI	National Cancer Institute
NOAEL	no observed adverse effect level
OBD	optimal biological dose
OTC	over-the-counter
PCR	polymerase chain reaction
PT	prothrombin time
PVG	pharmacovigilance
qPCR	quantitative polymerase chain reaction
QTcF	QT interval corrected by Fridericia's formula
SAE	serious adverse event
SAP	statistical analysis plan
SUSAR	suspected, unexpected, serious adverse reactions

Abbreviation	Definition
TEAE	treatment-emergent adverse event
ULN	upper limit of normal

1 Introduction

Hemophilia B is an X-linked recessive bleeding disorder that affects approximately 1 in 20,000 to 25,000 male births. Mutations in the factor IX (FIX) gene, which encodes for a serine protease produced by the liver and is critical for fibrin clot formation, lead to deficiencies in coagulation. The disease is characterized by frequent, spontaneous internal bleeding that can lead to chronic arthropathy (joint damage), intracranial hemorrhage, and even death. In patients with moderate/severe to severe hemophilia B, 90% of all bleeding episodes occur in the joints and, if not treated properly, lead to debilitating damage and a decreased quality of life [Simpson 2012]. Depending on the residual activity of FIX, disease severity is classified as mild (>5% to <40% of normal), moderate (1% to 5% of normal), or severe (<1% of normal) [White 2001]. The current standard of care for hemophilia B is intravenous (IV) injections of either recombinant or plasma-derived FIX concentrates at the time of a bleed (on-demand therapy) or prophylactically to prevent bleeding [Peyvandi 2013]. The current standard of care is a reasonable means to prevent and arrest hemorrhaging; however, there remains a high burden of use for these life-long therapies and current treatment options do not completely prevent the chronic and debilitating damage to joints and soft tissues that follows a bleeding episode.

Although the administration of these recombinant and plasma-derived FIX concentrates has significantly improved the clinical management of hemophilia B [Kaufman 2013], their use is limited in several ways:

1. The majority of recombinant FIX products have a short half-life, requiring IV infusions 2 to 3 times per week to maintain circulating FIX levels above 1% of normal activity, which is effective for preventing spontaneous bleeding episodes [Nilsson 1992]. Recently approved recombinant coagulation FIX concentrates indicated for hemophilia B still require IV injections approximately once weekly or every 10 days for routine prophylaxis, and per label stipulate that dosing regimen should be adjusted based on individual response [Alprolix™ Prescribing Information 2014];

2. Replacement therapy, including recently approved concentrates, does not provide constant, clinically important FIX activity levels. Patients on replacement therapy still experience FIX trough levels between dosing regimens that place them at risk for spontaneous bleeding, leading to chronic and debilitating arthropathy, intracranial hemorrhage, and even death;

3. Neutralizing antibodies (inhibitors) occur in up to 3% of patients with hemophilia B, rendering replacement FIX therapy ineffective [Di Michele 2007]. Recent publications have suggested that FIX production through gene therapy is unlikely to result in neutralizing antibodies and may even reverse existing immune response in patients with pre-existing inhibitors developed in response to replacement therapy [Niemeyer 2009; Scott 2012; Crudele 2015];

4. Current treatment options do not adequately prevent the chronic damage to joints and soft tissue associated with breakthrough bleeding episodes; and

5. When used prophylactically, only approximately 20% of the world's hemophilia population has access to these treatments [Mingozzi 2011].

There remains a significant unmet medical need for a less invasive treatment option for sustained management and prevention of bleeding episodes associated with hemophilia B.

Factor IX gene therapy is expected to be effective for hemophilia B because the disease is caused by the lack of the single gene product, FIX [Kay 2011]. The continuous synthesis of FIX by transduced liver cells is not expected to have the trough levels associated with prophylactic or on-demand FIX replacement therapy [Collins 2011]. Factor IX gene therapy is likely to promote the normal coagulation process and is expected to be more effective at preventing bleeds in patients with moderate to severe hemophilia B than repeated infusions of recombinant FIX. Factor IX gene therapy would also allow patients to avoid the risks and inconvenience of regular IV infusions. The overall reduction in bleeding frequency will have a positive impact on secondary effects, such as joint damage and improving the patient's quality of life.

1.1 Adeno-Associated Viral Vectors

Adeno-associated virus (AAV) is a non-enveloped, icosahedral, single-stranded DNA virus. Given that wild-type AAV displays wide tissue tropism and is capable of persisting in tissues for long durations without pathogenic effects, the use of recombinant AAV vectors has become a popular tool for gene delivery. Additionally, recombinant AAV vectors are non-replicating and the vector genomes exist as an episome following tissue transduction, minimizing the risk of insertional mutagenesis [Nakai 2001]. Since the first genetic engineering of wild-type AAV as a gene delivery vector in the early 1980s, recombinant AAV has shown great promise as an effective and safe gene delivery vehicle for treatment of diseases [Gao 2005].

Adeno-associated virus serotype 2 (AAV2) was the first AAV that was used for gene transfer applications and has been used in numerous studies for a variety of diseases such as alpha 1-antitrypsin deficiency, Batten disease, and cystic fibrosis [Mingozzi 2011]. Specifically, animal models for hemophilia B have shown that AAV-mediated delivery of FIX can treat the disease for many years [Snyder 1999; Mount 2002; Wang 2005; Nichols 2010]. However, similar results have not been recapitulated in humans, where the expression of FIX from AAV2-mediated delivery was either sub-therapeutic or only lasted a few months [Manno 2003; Manno 2006]. This was due to several limitations of AAV2 vectors including low transduction efficiency [Yan 2002], high seroprevalence of neutralizing antibodies against AAV2 in humans [Boutin 2010], and potentially destructive T-cell responses to capsids [Gao 2009; Vandenberghe 2006; Wang 2007]. There has also been evidence of B-cell responses against the transgene product in certain indications [Mingozzi 2011].

1.2 Selection of the AAV Clinical Candidate

The host immune response (ie, neutralizing antibodies and T-cell responses) limiting the efficacy of AAV2-mediated gene transfer (Section 1.1) was not predicted in the initial animal studies, due to AAV2 being endemic to humans and not these other species. Novel AAV serotypes, isolated from nonhuman primates, are divergent enough from endemic human serotypes to circumvent the neutralizing antibodies existing in most human subjects while retaining similar tissue tropism [Gao 2002].

One of these serotypes, AAV serotype 8 (AAV8), displays strong tropism for the liver [Gao 2002] and has been tested extensively in nonclinical and clinical models of hemophilia [Davidoff 2005; Jiang 2006; Nathwani 2006; Nathwani 2007; Nathwani 2011a]. These studies have shown that AAV8 has clear advantages over AAV2 including: excellent transduction efficiency; liver-specific tropism; stable transgene expression; a lack of hepatotoxicity as measured by peak serum transaminases; a lack of liver histopathology; a lack of T-cell activation to the transgene product; and low levels of pre-existing neutralizing antibodies in human populations, minimizing their inhibition of in vivo transduction.

Given the clinical experience with AAV8 in gene therapy studies for hemophilia B, adeno-associated virus serotype rh10 (AAVrh10), which shares 93.5% homology with AAV8, was pursued. Adeno-associated virus serotype rh10, as a clade E AAV, has strong tropism for the liver and transduces the liver highly efficiently when administered intravenously [Gao 2003; Gao 2004] and has shown impressive efficacy and safety in multiple pre-clinical animal models, such as Batten disease, Sanfilippo syndrome type A (mucopolysaccharidosis IIIA), and metachromatic leukodystrophy. Additionally, several US Food and Drug Administration (FDA)-approved Phase I clinical studies using AAVrh10 are ongoing or have recently completed enrollment (refer to ClinicalTrials.gov Identifiers: NCT01161576, NCT01414985, NCT01474343, and NCT01801709).

DTX101, the study product, is AAVrh10FIX, a non-replicating recombinant AAVrh10 encoding human FIX (hFIX) for the treatment of patients with hemophilia B. DTX101 encodes a codon-optimized hFIX gene with expression driven by both a liver-specific enhancer element and a liver-specific promoter element encapsulated within AAVrh10.

The proposed clinical study differs from previous hemophilia B AAV-mediated gene therapy approaches in 3 important aspects:

1. Adeno-associated virus serotype rh10 demonstrates high liver tropism and can achieve efficient liver gene transfer following IV infusion;

2. Adeno-associated virus rh10 is not thought to be endemic in the human population and, therefore, the prevalence of neutralizing antibodies to AAVrh10 in hemophilia B patients is expected to be lower than other AAV serotypes, such as AAV2;

3. DTX101 will be manufactured using a process that will result in the generation of fewer empty particles (ie, particles lacking the FIX transgene). This is anticipated to achieve a clinically meaningful rise in FIX activity, while potentially resulting in less frequent or less severe adverse events (AEs) than noted with other AAV preparations.

1.3 Study Rationale

1.3.1 Design Rationale

The design of this study is consistent with global regulatory guidelines for protocol design, including: subject selection, dose estimation, precautions applied between dosing cohorts, risk mitigation, and study stopping criteria.

Study 101HEMB01 is a Phase I/II, open-label, single-arm, multicenter, dose-finding safety study to determine the safety, tolerability, and efficacy of DTX101 in adult males with moderate/severe to severe hemophilia B. The primary objectives of the study are to determine the safety of single ascending IV doses of DTX101 and to identify the optimal biological dose (OBD) of DTX101 that either achieves or is the closest to achieving the target peak FIX activity of ≥20% of normal.

Eligible subjects will receive a single IV infusion of DTX101. A minimum of 3 subjects will be enrolled per cohort. Subjects within a cohort will be dosed at a minimum of 1 week apart. Dose escalation will be conducted according to a model that uses both safety and efficacy data to predict the OBD (Section 10.5.1).

A Data Safety Monitoring Committee (DSMC) will meet after each dosing cohort to review subject safety (eg, AEs, physical examination findings, vital sign measurements, electrocardiogram [ECG] results, and clinical laboratory assessments including assessment of liver function) and provide their recommendation for progressing to the next dosing level (Section 9.3).

Subjects will be followed for 52 weeks after dosing. After completion of this study, subjects will be offered enrollment in an extension study to evaluate the long-term safety and effect of DTX101 on clinical outcome measures.

1.3.2 Dosing Rationale

The nonclinical pharmacology of a single IV dose of DTX101 in FIX knockout and wild-type mice demonstrated selective distribution to the liver, effective gene expression, and predictable dose-related increases in FIX associated with expected physiological changes in the biomarkers of the intrinsic clotting cascade.

The nonclinical findings with DTX101 and research of peer-reviewed literature on the effect of FIX transgene expression in humans provides supports that DTX101 administered as a single IV dose to patients with moderate/severe to severe hemophilia B will promote the normal coagulation process and thereby, reduce the total number of annual bleeding episodes associated with the disease. Reduced bleeding frequency will also have a positive impact on secondary effects, such as joint damage. The clinical dosing rationale selects doses expected to provide maximal therapeutic benefit, with minimal risk to subject safety.

Based on the formal DTX101 minimal effective dose (MED) study (Study DTX150226; refer to Section 4.2.1.3 of the Investigator's Brochure [IB]), the MED of DTX101 in FIX knockout mice falls between 1.6×10^{10} to 5.0×10^{10} genome copies (GC)/kg, which produces between 4% and 34% of normal FIX levels, respectively. Previous nonclinical studies have estimated that a 10-fold higher dose of AAV vector is required in nonhuman primates to achieve similar expression levels as compared to mice (refer to Section 2.2 of the IB), however transduction rates of human hepatocytes are thought to be even lower. The most similar nonhuman primate to human comparison that is available for clade E AAV liver-directed gene therapy for hemophilia B is with scAAV8-FIX, a closely related vector to DTX101, and suggests that transduction and expression in humans is significantly lower than in nonhuman primates.

In nonclinical studies by Nathwani et al, rhesus macaques inoculated with scAAV8-FIX at a dose of 2.0×10^{11} GC/kg expressed a peak of 35% to 40% of normal FIX levels in serum by Day 15 [Nathwani 2011b]. It must be noted that later correction of dosage methods suggested these animals actually received a dose of 2.0×10^{12} GC/kg [Nathwani 2011a]. In comparison, when administered to human patients in a Phase I dose-escalation clinical trial, 2.0×10^{12} GC/kg resulted in sustained average levels of only 5.1±1.7% of normal FIX activity [Nathwani 2011a; Nathwani 2014]. Based on the corrected dosing, this represents approximately a 10-fold difference in transduction and expression levels between nonhuman primates and humans.

When considering a clinical dosing rationale, in light of the available comparison data between mice, nonhuman primates and humans, it seems reasonable to consider a 100-fold increase in dose from mice to humans to achieve similar target levels of FIX. Therefore, the proposed MED range for human dosing with a target peak expression level of 20% would fall between 1.6×10^{12} and 5.0×10^{12} GC/kg. The proposed initial clinical dose of DTX101 is 1.6×10^{12} GC/kg, 100-fold above the lower end of the MED range in the mouse. Dose escalation would follow at either 3.0×10^{12} GC/kg or 5.0×10^{12} GC/kg. The final dose, 1.0×10^{13} GC/kg, is consistent with a half log increase over the upper end of the proposed human MED range.

A starting dose of 1.6×10^{12} GC/kg has a safety margin of 3-fold versus the no observed adverse effect level (NOAEL) determined in the formal GLP toxicology study (Study 2371-002; refer to section Section 4.4.1.2 of the IB). The primary safety finding from this study was the prolongation of aPTT times that correlated with supraphysiological FIX expression and activity levels. The highest dose tested, 5.0×10^{13} GC/kg, resulted in a peak average prolongation of aPTT of 63% at Day 15. This prolongation began to resolve by Day 29, being 43% at Day 29 and 15% by Day 90. Mice in this dose group (5.0×10^{13}GC/kg) were expressing supraphysiological levels of FIX at more than 2500% of normal.

Although prolongation of aPTT was present, these animals exhibited no related clinical signs or complications. Lower doses of DTX101 resulted in minimal to moderate increases of aPTT that were often not statistically significant.

Unlike wild-type mice, FIX knockout mice dosed at 1.35×10^{14} GC/kg demonstrated a reduction in aPTT time from 74.3 seconds (average for FIX knockout mice) to 45 seconds (below the control group average of 50 seconds), likely due to the absence of endogenous FIX at baseline in the knockout mice (Study W2234; refer to Section 4.4.1.1 of the IB). This finding in the FIX knockout mouse, a model used to explore the human condition, is expected to more closely represent the clinical outcome in the target patient population of moderate/severe to severe hemophilia B patients. It is believed to be unlikely that the proposed starting clinical dose of 1.6×10^{12} GC/kg in a patient with moderate/severe to severe hemophilia B would achieve supraphysiological levels of over 2500% normal FIX expression. Therefore, it is not likely that associated prolongation of aPTT, as a direct result

of marked supraphysiological levels of FIX, will occur in the target population at the starting dose proposed.

The second and third dose candidates in this study have approximately a 1.7-fold and 1.0-fold safety margin, respectively. The fourth and final candidate dose in the study is 1.0×10^{13} GC/kg (Table 1–1).

Table 1–1 Predicted Total Vector Titer of DTX101 Following IV Infusion and Exposure Margins to a Nonclinical GLP Toxicology Study

Candidate Dosing Levels	DTX101 Dose (GC/kg)	Safety Margin[a]
Dose 1	1.6×10^{12}	3.1
Dose 2	3.0×10^{12}	1.7
Dose 3	5.0×10^{12}	1
Dose 4	1.0×10^{13}	–

Abbreviations: GC, genome copies; GLP, Good Laboratory Practice; IV, intravenous.
a. Mouse no observed adverse effect level of 5.0×10^{12} GC/kg / Human DTX101 dose.

Taking into consideration the nonclinical pharmacology of DTX101 in knockout mice and the NOAEL combined with the known differential efficiency of transgene uptake and expression between animals and humans, the proposed initial starting dose for DTX101 of 1.6×10^{12} GC/kg is assessed to offer an acceptable balance between safety and the potential for clinically meaningful FIX expression in moderately/severe and severe hemophilia B patients.

It has been hypothesized that the AEs of elevated liver function tests (LFTs) experienced to date in other clinical studies using AAV8-derived vectors were due to the total capsid protein load administered to subjects [Mingozzi 2011; Nathwani 2011a; Nathwani 2014]. Based on the manufacturing process used to prepare DTX101, it is estimated that >50% of the DTX101 study product will contain full particles (ie, particles that contain the FIX transgene). This reduces the total particle dose that each subject is exposed to with each increase in dose (Table 1–1). In a previous study with AAV8, a transient rise in ALT was

elicited at the highest dose tested, 2.0×10^{12} GC/kg, which is approximately 2.0×10^{13} particles/mL and contains 10% full particles [Nathwani 2011a; Nathwani 2014].

Because dosing is based on genome copies, the total particle amount (full capsids + empty capsids) for Dose 1 to Dose 3 is estimated to be lower than the total AAV8 particle amount used in other studies. The total particle amount at the highest dose of DTX101 (1.0×10^{13} GC/kg) is estimated to be similar or slightly lower than the total AAV8 particle amount previously studied [Nathwani 2011a; Nathwani 2014]. It is expected that the higher ratio of full to empty capsids may decrease or possibly eliminate the AE of elevated LFTs observed in other gene therapy programs and increase the benefit-to-risk ratio for subjects participating in this study.

For additional nonclinical information, please refer to the DTX101 IB.

2 Study Objectives and Endpoints

Objective	Endpoint
Primary	
To determine the safety of single ascending intravenous (IV) doses of DTX101 in adults with moderate/severe to severe hemophilia B.	The incidence of adverse events (AEs), treatment-emergent adverse events (TEAEs), and serious AEs will be summarized for each dosing cohort by severity and relationship to study product.
To establish a dose of DTX101 that achieves a peak plasma level of vector-derived factor IX (FIX) at 6 weeks after IV administration to allow further clinical development.	The change from baseline in FIX activity at Week 6 as determined by the activated partial thromboplastin time (aPTT) clot-based assay.
Secondary	
To assess the impact of DTX101 on the number of bleeding episodes requiring recombinant FIX infusion during the study.	The annualized bleeding rate (ABR) will be calculated for all subjects through Week 52 (±7 days).
To evaluate the kinetics, duration, and magnitude of plasma FIX activity, by dose, after IV administration of DTX101 in adults with hemophilia B.	The time course of FIX activity, as determined by aPTT, will be summarized by time point and dose level of DTX101.
To assess the impact of DTX101 on the frequency of FIX replacement therapy during the study.	The annualized and average weekly use of FIX replacement therapy will be calculated for all subjects through Week 52 (±7 days).
To describe the immune response to the FIX transgene after IV administration of DTX101.	• The development of neutralizing antibodies to FIX (FIX inhibitor), as determined by a Bethesda assay, will be summarized by time point and dose level of DTX101. • The development of a cell-mediated immune response to FIX, as determined by ELISPOT assay, will be summarized by time point and dose level of DTX101.
To assess the impact of DTX101 on the subject's quality of life	Responses to the EQ-5D-5L and Haem-A-QoL questionnaires will be summarized.

Objective	Endpoint
Exploratory	
To describe the immune response to AAVrh10 capsid proteins after IV administration of DTX101.	• The development of neutralizing antibodies to AAVrh10, as determined by ELISA, will be summarized by time point and dose level of DTX101. • The development of a cell-mediated immune response to AAVrh10, as determined by ELISPOT assay, will be summarized by time point and dose level of DTX101. • The development of anti-AAVrh10 binding antibodies, as determined by ELISA, will be summarized by time point and dose level of DTX101.

Abbreviations: AAVrh10, adeno-associated virus serotype rh10; ABR, annualized bleeding rate; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; EQ-5D-5L, EuroQoL 5D 5 level; Haem-A-QoL, haemophilia-specific quality of life.

3 Investigational Plan

3.1 Study Overview

Study 101HEMEB01 is a Phase I/II, open-label, single-arm, multicenter, dose-finding safety study to determine the safety, tolerability, and efficacy of DTX101 in adults with moderate/severe to severe hemophilia B. The primary objectives of the study are to determine the safety of single ascending IV doses of DTX101 and to identify the OBD of DTX101 that either achieves or is the closest to achieving the target peak FIX activity of ≥20% of normal.

Eligible subjects will receive a single IV infusion of DTX101. Subjects will be dosed sequentially in cohorts with a minimum of 3 subjects each. Dose escalation will be conducted according to a model that uses safety and efficacy data to predict the OBD (Section 10.5.1).

The following candidate doses (per kg of body weight) will be used to identify the OBD:

- **Dose 1**: 1.6×10^{12} GC/kg
- **Dose 2**: 3.0×10^{12} GC/kg
- **Dose 3**: 5.0×10^{12} GC/kg
- **Dose 4**: 1.0×10^{13} GC/kg

There will be a minimum of 7 days between dosing of each subject within a cohort and a minimum of 42 days between the dosing of the last subject in one dosing cohort and the first subject in the next dosing cohort. The recommendation to proceed to the next dose cohort will be made by the DSMC (Section 9.3) after evaluation of the safety data for all subjects in a dosing cohort after they have completed Week 6, as outlined in the DSMC charter. There will be no intra cohort dose escalations.

Study enrollment will be suspended if any of the safety stopping criteria are met (Section 3.2.4).

Subjects will be followed for 52 weeks after dosing. After completion of this study, subjects will be offered enrollment in an extension study to evaluate the long-term safety and effect of DTX101 on clinical outcome measures.

3.2 Overall Study Duration and Follow-Up

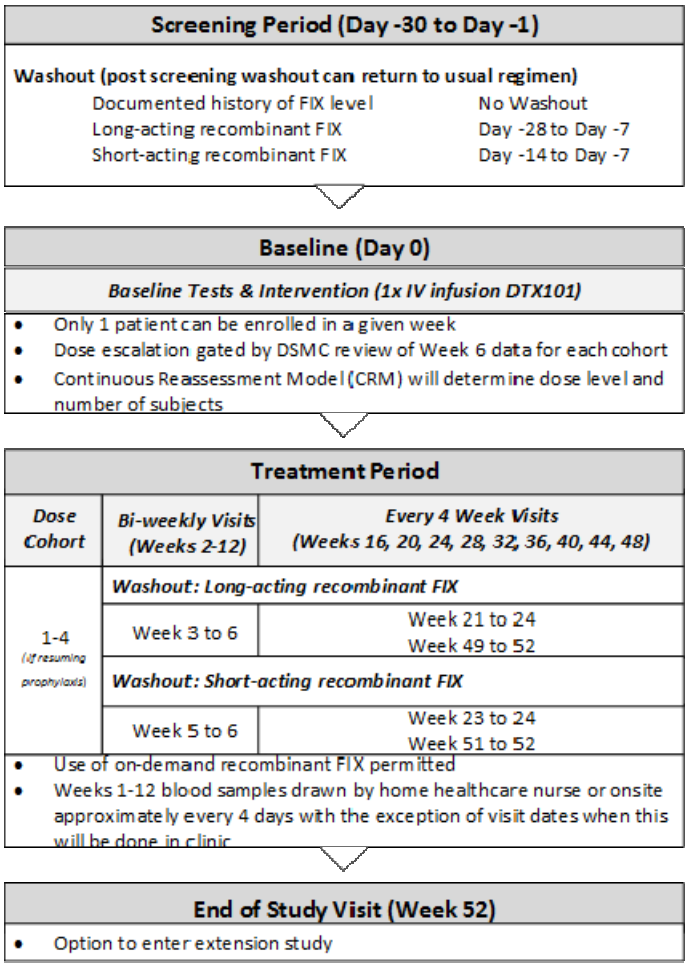
The duration of the study is defined for each subject as the date written informed consent is provided through the visit at Week 52. Subjects will be in the study for approximately 56 weeks (including the screening period).

The study is anticipated to enroll approximately 12 to 18 subjects at up to 14 sites globally.

Subjects will be asked to visit the study site a total of approximately 30 times; however, if a subject cannot visit the site for every visit, they may be visited at home through Week 12 for sample collection (ie, clinical chemistry and viral shedding). All study visits, clinic or home visits, and the timing of assessments are detailed in Table 15–1 and Table 15–2.

A schematic of the study design is provided in Figure 3–1.

Figure 3–1 Study Design



Abbreviations: DSMC, Data Safety Monitoring Committee; FIX, factor IX; IV, intravenous.

3.2.1 Screening Period

After a subject has provided written informed consent, and within 30 days before DTX101 infusion (Day 0), the investigator or other qualified study personnel will determine if the subject is eligible for the study. This will be accomplished by reviewing the inclusion and exclusion criteria and completing all of the screening assessments outlined in the Schedule of Events (Table 15–1). The screening assessments may be performed on more than 1 day, provided that all of the assessments are completed and the results are available within the 30-day screening window and prior to Day 0.

3.2.2 Treatment Period

Subjects will be admitted for DTX101 administration and for continuous safety monitoring (Section 8.2). Subjects will be discharged 24 hours after completion of the infusion.

On Day 0, subjects will be provided a paper or an electronic diary (eDiary), depending on availability, to record bleeding episodes and use of recombinant FIX replacement therapy during the study. The diary will be reviewed with the subject at each visit.

Subjects will be asked to visit the clinic every 4 days through Week 12. Following the Week 12 visit, subjects will visit the study site once every 4 weeks through Week 52 or early withdrawal from the study.

Subjects will be asked to provide clinical laboratory samples approximately every 4 days through Week 12 of the study. If subjects cannot visit the study site in person, subjects can be seen by clinically trained and qualified personnel approximately every 4 days at their home through Week 12, the exception being those weeks when a site visit is scheduled, to collect a clinical laboratory sample to monitor LFTs. Saliva, urine, and stool samples will also be collected for assessment of viral shedding on Days 8, 20, 36, 48, 64, and 76. These samples will continue to be collected until 3 consecutive negative results are obtained from each sample matrix (Table 15–2).

3.2.3 Prophylactic Factor IX Washout Periods

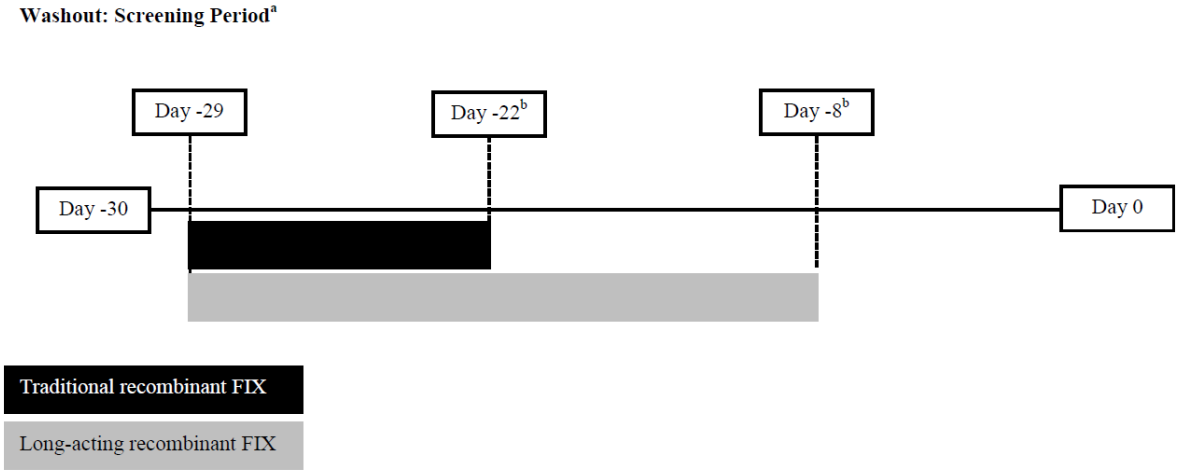
3.2.3.1 Screening Period

If the subject does not have a documented history of FIX activity, a blood sample will be needed to confirm the severity of hemophilia B for inclusion (Section 4.1) after washout of recombinant FIX (Figure 3–2). For subjects taking long-acting recombinant FIX prophylactically to prevent bleeding episodes, the washout period is to start at approximately Day –29 and last for 21 days (approximately Day –8). For subjects taking traditional recombinant FIX prophylactically to prevent bleeding episodes, the washout period is to start at approximately Day –29 and last for 7 days (approximately Day –22). The blood sample must be obtained and results available prior to dosing on Day 0. Subjects can then transition

back onto their prophylactic regimen until the washout period during the treatment period (prior to Week 6) is to begin (Section 3.2.3.2).

NOTE: This washout period is not needed for subjects with a documented history of FIX activity.

Figure 3–2 Recombinant FIX Washout During the Screening Period



Abbreviations: FIX, factor IX.

a. Washout required for subjects that do not have a documented history of FIX activity to meet eligibility criterion (ie, ≤2% of normal).

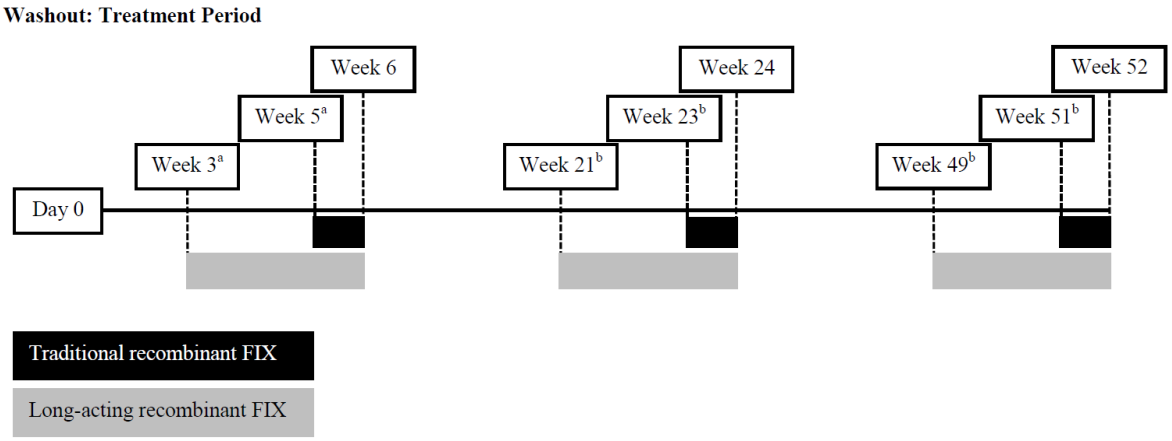
b. Following assessment of FIX activity, subjects can transition back on to their prophylactic regimen until the protocol mandated washout period prior to the Week 6 assessment of FIX activity.

3.2.3.2 Treatment Period

In order to accurately assess vector-derived FIX activity as a result of DTX101 infusion, subjects will stop taking FIX prophylactically prior to the assessment of peak FIX activity at Week 6 and transition to an on-demand regimen for the remainder of the study. Subjects prophylactically using traditional recombinant FIX will be asked to begin the washout period starting at Week 5. Due to the longer half-life, subjects prophylactically taking long-acting recombinant FIX (eg, Alprolix) will be asked to begin the washout period starting at Week 3 (Figure 3–3).

If, at any time during the study, a subject experiences bleeding episodes frequent enough to consider prophylactic treatment, the investigator will discuss treatment options with the medical monitor. If a subject resumes prophylactic FIX replacement therapy, as rescue treatment after Week 6, additional washout periods prior to Week 24 and Week 52 will also be performed. Subjects prophylactically using traditional recombinant FIX will be asked to begin the washout period starting at Week 23 and Week 51. Due to the longer half-life, subjects taking long-acting recombinant FIX (eg, Alprolix) will be asked to begin the washout period starting at Week 21 and Week 49 (Figure 3–3).

Figure 3–3 Recombinant FIX Washout During the Treatment Period



Abbreviations: FIX, factor IX.

a. Following washout, subjects will be transitioned to an on-demand regimen for the remainder of the study.
b. Washout needed if, at any time during the study after Week 6, a subject experiences bleeding episodes frequent enough to consider re-initiating prophylactic treatment.

The on-demand use of recombinant FIX is not prohibited and may be used at any time during the study. Subjects will be asked to record this use as outlined in Section 8.1.3.

3.2.4 Safety Stopping Criteria

Enrollment will be suspended and the study will be discussed with regulators if, at any time during the study, any of the following occur:

- Death of a subject, at any time, that is considered related to DTX101 by the investigator,
- Fivefold increase in baseline (predose, Day 0) ALT within 8 weeks after vector infusion that is considered related to DTX101 by the investigator,
- Grade 2 toxicity develops that persists for more than 7 days and is considered related to DTX101 by the investigator,
- At least Grade 3 toxicity develops, including an infusion-related reaction ≥Grade 3, that is considered related to DTX101 by the investigator, or
- Occurrence of a malignancy, at any point after administration of vector, that is considered related to DTX101 by the investigator.

Abnormal clinically significant laboratory values (hematology, coagulation panel, and clinical chemistry) as assessed by the investigator will be considered AEs. Laboratory values that meet any of the above criteria will result in the suspension of the study.

Any event that meets the above criteria will be reported immediately as outlined in Section 9.1.2.2, and the appropriate pages of the electronic case report form (eCRF) must be completed. Enrollment and dosing will be temporarily suspended until the situation can be assessed and risks to subjects mitigated. If study enrollment is suspended, all subjects who have been enrolled will remain in the study and will continue to be monitored through their completion or withdrawal from the study.

3.2.5 End of Study

Subjects who complete all visits up to and including the Week 52 visit will have completed the study. Subjects who discontinue early will be asked to return for an Early Withdrawal visit. Subjects with any on-going AEs at this visit will continue to be monitored as outlined in Section 9.1.5. After completion of this study, subjects will be offered enrollment in an

extension study to evaluate the long-term safety and effect of DTX101 on clinical outcome measures.

4 Subject Selection

Subjects will be assigned to study treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

4.1 Inclusion Criteria

Each subject must meet all of the following criteria at screening to be enrolled in this study:

1. Male ≥18 years of age.
2. Moderate/severe or severe hemophilia B (baseline FIX activity ≤2% of normal or documented history of FIX activity ≤2%).
3. At least 3 bleeding episodes per year that require on-demand treatment with FIX OR are treated with a prophylactic regimen of FIX.
4. At least 100 days exposure history to FIX.
5. No documented history of inhibitors (neutralizing antibodies) to exogenous FIX.
6. No known allergic reaction to exogenous FIX or any component of DTX101.
7. Willing to stop prophylactic treatment with recombinant FIX at specified time points during the study.
8. Willing and able to provide written informed consent.
9. Willing and able to comply with study procedures and requirements.
10. Willing to use effective contraception at the time of administration of DTX101 and for 3 months following administration of DTX101. Appropriate contraceptive methods include a condom with spermicide. Abstinence, defined as sexual inactivity, is an acceptable form of birth control; however, appropriate contraception must be used if the subject becomes sexually active.

4.2 Exclusion Criteria

Subjects who meet any of the following criteria at screening will be excluded from the study:

1. History of liver disease as evidenced by any of the following: portal hypertension, ascites, splenomegaly, esophageal varices, hepatic encephalopathy, or a liver biopsy with evidence of stage 3 fibrosis.
2. Significant hepatic inflammation or cirrhosis as evidenced by any of the following: aspartate aminotransferase (AST) or ALT >2.0 × upper limit of normal (ULN), total bilirubin >1.5 × ULN, alkaline phosphatase (ALP) >2.5 × ULN, platelet count <75,000 cells/μL, and prothrombin time (PT) or international normalized ratio (INR) >1.5 × ULN.
3. Evidence of active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, documented by current use of antiviral therapy for HBV or HCV or by hepatitis B surface antigen (HBsAg) or HCV RNA positivity. NOTE: Two negative viral assays by polymerase chain reaction (PCR), collected at least 6 months apart, will be required to be considered negative for HCV. Subjects can be rescreened once if they have one negative sample and must wait to have the second sample collected within the following 6 months.
4. History of human immunodeficiency virus (HIV) infection AND any of the following: CD4+ cell count <350 cells/mm^3, change in antiretroviral therapy regimen within 6 months prior to Day 0, or plasma viral load >200 copies/mL, on 2 separate occasions, as measured by PCR.
5. Anti-AAVrh10 neutralizing antibody titer >1:5.
6. Participation (current or previous) in another gene therapy study.
7. Participation in another investigational medicine study within 3 months before screening.
8. History of a malignancy for which the subject has received treatment in the past 2 years except for prostate cancer treated with watchful waiting or surgically removed non-melanoma skin cancer.

9. Has any other significant medical condition that the investigator feels would be a risk to the subject or would impede the study.

5 Screening and Randomization Procedures

5.1 Subject Screening

All potential subjects will sign an informed consent form (ICF) before any study procedures or the washout period (if needed) are performed or initiated (Section 11.3). Subjects will have the opportunity to have any questions answered before signing the ICF. All questions raised by the subject must be addressed before the investigator also signs the ICF. A copy of the signed consent will be given to the subject.

Subjects can be rescreened once if they have 1 negative HCV viral assay by PCR at the time of screening and must wait 6 months for receipt of the second sample result. NOTE: To be eligible to participate in the trial, subjects must have 2 negative HCV viral assays by PCR at least 6 months apart.

Sites will maintain documentation of all potential subjects screened for inclusion in the study. If a subject is excluded from the study, the reasons for exclusion will be documented in the subject's source documents and on the screening log.

5.2 Subject Randomization

This is an open-label study; subjects will be enrolled sequentially into cohorts of a minimum of 3 subjects each as described in Section 3.1.

6 Study Treatment

6.1 Identity of Study Product

6.1.1 Description of DTX101

DTX101 is a replication defective, recombinant AAV vector of the rh10 serotype. It is a thermally stable recombinant parvovirus [Rayaprolu 2013]. DTX101 is supplied in a hyperosmotic buffered formulation solution of approximately 400 milliosmole and at pH 8.0. DTX101 is a homogeneous, monodisperse solution that is clear and colorless without visible particulates.

6.1.2 Components Used for Manufacturing

DTX101 will be produced by triple plasmid DNA transfection of human embryonic kidney 293 (HEK293) master cell bank cells with the following:

- The pDTX.hFIX.101 vector plasmid;
- An AAV helper plasmid termed pAAV2.rh10.KanR containing the AAV rep2 and cap AAVrh10 wild-type genes; and
- A helper adenovirus plasmid termed pAdDeltaF6(Kan).

The size of the DTX101 packaged vector genome is 3972 nucleotides. For additional information, please refer to the DTX101 Investigator's Brochure.

6.2 Management of Clinical Supplies

6.2.1 Packaging and Labeling

One (1) mL of study product will be provided to study sites frozen in 2 mL sterile glass vials with a primary label on the vial. Study product will have secondary packaging with a secondary label. The primary label meets all requirements for blister and small packaging units and will contain a unique identifier. The secondary label will contain required text for

all countries participating in the study and will also contain a unique identifier. Secondary labeling will appear in the appropriate language for the country supplied.

6.2.2 Storage of DTX101

DTX101 must be stored in a secure freezer at a controlled temperature ≤–60°C. The site is to maintain a daily log documenting the temperature.

6.2.3 Study Product Accountability

The investigator or designated personnel will maintain accurate records of receipt of all study product, including dates of receipt. In addition, accurate records will be kept regarding when and how much study product is dispensed and used by each subject in the study. Reasons for departure from the expected dispensing regimen must also be recorded. At the completion of the study, all study product will be reconciled and retained or destroyed according to instructions provided by the sponsor.

6.2.4 Transmission of Infectious Agents

Recombinant AAV vectors are non-replicative and are not expected to pose a risk of transmission. However, all sexually active male subjects must use approved contraception from the time of dosing and for 3 months following. The study product and post-treatment study samples should be handled using standard universal precautions.

6.3 Treatment Schedule and Administration

Subjects will receive a single peripheral IV infusion of DTX101, administered by qualified study personnel as designated by the investigator. The dose will be determined by the cohort and candidate dose (Section 3.1).

The dose of DTX101 to be administered will be calculated using the subject's weight recorded at screening. The subject's weight will be verified prior to administration of DTX101 to ensure that their current weight is within 10% of their screening weight. Prior to infusion, all infusion bag labels will be checked by the site pharmacist and a minimum of 2 medical personnel charged with administration of DTX101.

The study site must be equipped with emergency resuscitation capabilities. The subject will be admitted on the day of infusion (Day 0). An IV catheter will be inserted into a peripheral vein and flushed with saline. Recombinant FIX treatment is not needed as venipuncture is a minimally invasive procedure.

Detailed instructions for dose preparation and subsequent infusion of DTX101 are provided in the pharmacy manual.

6.3.1 Treatment Compliance

DTX101 will be administered at the infusion center via a single IV infusion administered by qualified personnel. The dose, start time, stop time, and volume of infusion will be recorded in the subject's eCRF.

6.4 Prior and Concomitant Therapy

Use of all prior and concomitant medications will be recorded in the subject's eCRF. The minimum requirement is that the drug name, the dates of administration, and the reason for use are to be recorded. This will include all prescription drugs, herbal products, vitamins, minerals, and over-the-counter (OTC) medications. Any changes in concomitant medication use will also be recorded in the subject's eCRF.

6.4.1 Permitted Medications

The use of permitted medications (date, dosage, reason for therapy) will be recorded on the relevant page of the eCRF.

6.4.1.1 Medications for the Treatment of Hemophilia B

- On-demand recombinant FIX replacement therapy, as needed;
- Standard supportive care medications for the treatment of symptoms associated with a bleeding episode.

6.4.1.2 Other Medications

If a subject starts a new medication, including OTC medications and herbal supplements, it should be discussed with the investigator.

Any concomitant medication deemed necessary for the welfare of the subject during the study may be given. It is the responsibility of the investigator to ensure that details regarding the medication are recorded in full in the eCRF.

6.4.2 Prohibited Medications

Use of any of the following medications is prohibited, unless the investigator feels these medications are medically indicated. If medically indicated, the use of prohibited medications (date, dosage, reason for therapy) will be recorded on the relevant page of the eCRF:

- Acetylsalicylic acid or nonsteroidal anti-inflammatory drugs;
- Another investigational product to treat hemophilia B;
- Another gene therapy product;
- Any other medication currently under investigation; or
- Immunosuppressive agents except steroids.

7 Withdrawal of Subjects From the Study

7.1 Study Withdrawal

Subjects may withdraw from the study at any time and for any reason without prejudice to their future medical care by the investigator or at the study site. Any subject who withdraws consent to participate in the study will be removed from further treatment and/or study observation immediately upon the date of request.

The investigator must record the reason for withdrawal on the relevant page of the eCRF. The reason for withdrawal may include the following:

1. Withdrawal of consent
2. Administrative decision by the investigator or the sponsor
3. Ineligibility
4. Significant protocol deviation
5. Subject noncompliance
6. Adverse event

If a subject is withdrawn due to an AE, the investigator will arrange for the subject to have follow-up visits until the AE has resolved or stabilized (Section 9.1.5).

If a subject requests or decides to withdraw from the study, all efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal, and an early withdrawal visit will be performed.

7.2 Subject Replacement

If a subject withdraws from the study after receiving DTX101, the subject will not be replaced. Subjects who withdraw from the study after signing the ICF, but before receiving DTX101, will be replaced and the replacement subject will be sequentially assigned to treatment with a new subject identification number.

8 Study Assessments and Procedures

8.1 Efficacy and Pharmacodynamic Assessments

Planned time points for all efficacy and pharmacodynamic measurements in the study are listed in Table 15–1. Efficacy will be assessed based on the change from baseline in FIX activity, the number of bleeding episodes, and the dosing requirements for FIX replacement therapy.

8.1.1 Factor IX Activity

The change from baseline in FIX activity at Week 6 and at additional time points throughout the study will be assessed. Factor IX activity will be determined using an aPTT clot-based assay. Planned time points for the assessment of FIX activity are listed in Table 15–1. Details for the preparation and shipment of samples are included in the laboratory manual.

8.1.2 Bleeding Episodes

Bleeding episodes will be recorded in a paper or eDiary and will include: the start/stop date, the location of the bleed (joint, muscle, skin/mucosa, or internal), and the type of bleed (spontaneous or traumatic). The start date of a bleeding episode is defined as the first onset of symptoms of a bleed. The stop date of a bleeding episode is defined as 72 hours after the last on-demand treatment for a bleed, during which no additional bleeding episodes were noted in that location. If a bleeding event occurs in the same location within 72 hours of on-demand therapy, it will be considered part of the same episode. The site will review the diary with the subject at each clinic visit and record bleeding episodes on the appropriate page of the eCRF. Any event considered an AE should be recorded on the appropriate page of the eCRF (Section 9.1.2).

The annualized number of bleeds per subject, per location, and by type of bleed will be calculated for all subjects through Week 52 (±7 days).

8.1.3 Factor IX Replacement Therapy

The use of on-demand FIX replacement therapy will be recorded in a paper or eDiary and will include: the date(s) and dose (IU/kg) administered. The site will review the diary with the subject at each clinic visit and record the use of FIX replacement therapy on the appropriate page of the eCRF.

The annualized and average weekly use of FIX replacement therapy will be calculated for all subjects through Week 52 (±7 days).

8.1.4 Quality-of-Life Assessments

8.1.4.1 EuroQol 5D 5 Level Questionnaire

Subjects will be asked to complete the EuroQol 5D 5 level (EQ-5D-5L) questionnaire (Appendix 15.2.1) on Day 0 (predose) and then at Weeks 24, 36, 48, and 52 during the study (Table 15–1). If the subject is provided an eDiary, this questionnaire will be preloaded onto the device. If either the eDiary or a validated translation for the eDiary is not available, the subject will be provided with a paper form to complete.

This questionnaire consists of 2 pages: the EQ-5D™ (EuroQol, Rotterdam, The Netherlands) descriptive system and the EQ visual analogue scale (EQ-VAS). The descriptive system is based on 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety depression. Each dimension has the following 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems. The EQ-VAS will record the subject's self-rated health on a vertical visual analogue scale. The results will be transmitted for analysis following completion of the Week 52 visit.

8.1.4.2 Haemophilia-Specific Quality of Life Questionnaire

Subjects will be asked to complete the Haem-A-QoL questionnaire (Appendix 15.2.2) on Day 0 (predose) and then at Weeks 24, 36, 48, and 52 during the study (Table 15–1). If the subject is provided an eDiary, this questionnaire will be preloaded onto the device. If either the eDiary or a validated translation for the eDiary is not available, the subject will be provided with a paper form to complete.

This questionnaire asks subjects about their perceptions of their health and treatment. The questionnaire is divided into the following 10 dimensions: physical health, feelings, view of themselves, sports & leisure, work & school, dealing with hemophilia, treatment, future, family planning, and partnership & sexuality. Questions are based on a 5-point Likert-scale (1=never, 2=rarely, 3=sometimes, 4=often, 5=all the time). If the question does not apply to the subject, the "not applicable" response is allowed in 3 of the domains (sport & leisure, work & school, family planning). Positively worded items need to be re-coded and domains will be transformed ranging from 0 to 100; higher domain and total scores indicating a higher impairment of health-related QoL. The results will be transmitted for analysis following completion of the Week 52 visit.

8.2 Safety Assessments

Planned time points for all safety assessments are listed in the Schedules of Events (Table 15–1 and Table 15–2).

Safety will be assessed based on AEs, serious adverse events (SAEs), complete and targeted physical examination findings (including weight), vital sign measurements, ECG results, clinical laboratory assessments (hematology, clinical chemistry, coagulation panel, and urinalysis), viral shedding, measurement of neutralizing antibody titer to AAVrh10, measurement of AAVrh10 binding antibodies, measurement of FIX inhibitor, and assessment of any cell-mediated immune responses to AAVrh10 and FIX.

8.2.1 Demographic, Medical, and Hemophilia B History Assessments

As allowed by local laws and regulations, the following demographic data will be captured on the appropriate page of the eCRF: date of birth, sex, race, and ethnicity.

Medical, medication, and hemophilia B history will be assessed as related to the eligibility criteria listed in Section 4.1 and Section 4.2.

8.2.2 Physical Examination

A complete physical examination will include assessments of the head, eyes, ears, nose, and throat; skin; and the endocrine metabolic, neurological, respiratory, cardiovascular, gastrointestinal, and musculoskeletal systems.

A targeted physical examination will include assessment of the skin and the respiratory, cardiovascular, and gastrointestinal systems.

Physical examination findings will be captured on the appropriate page of the eCRF.

8.2.3 Vital Sign Measurements

During the study, vital sign measurements are to be collected before any stimulating or anxiety-provoking procedures (eg, phlebotomy). Vital sign measurements will include heart rate, blood pressure (systolic and diastolic), and respiratory rate. Height (at the screening visit only) and weight will also be recorded.

- Vital signs should be measured with the subject in a semi-supine or supine position, having rested in this position for at least 5 minutes beforehand. It is preferred that the measurement be late with the subject rested, rather than on-time with the subject not sufficiently rested. If the subject is not sufficiently rested, this needs to be stated in the source documents.
- On Day 0, vital signs will be measured at predose, 5 minutes, and 0.5, 1, 2, 4, 6, and 8 hours after the start of infusion (±5 minutes). Vital signs will also be measured at 22 hours (±1 hour) after the start of infusion, prior to subject discharge.
- Blood pressure and heart rate will be recorded in triplicate before dosing, with measurements taken at least 2 minutes apart.
- It is acceptable for heart rate to be captured from the 12-lead ECG.
- On Day 0, weight will be measured prior to dosing with DTX101 to ensure that the weight is within 10% of the screening weight used to calculate the dose of DTX101.

Vital sign measurements will be recorded on the appropriate page of the eCRF. The medical monitor should be notified of any clinically significant changes or abnormal value in vital sign measurements (Section 9.1.2).

8.2.4 Electrocardiograms

- Three ECG measurements will be taken before dosing, at least 5 minutes apart. The PR interval, QRS duration, QT interval, and QTcF (QT interval corrected by Fridericia's formula) will be obtained from automated ECG readings. The mean value obtained before dosing on Day 0 will be classified as baseline;
- A single 12-lead ECG will be obtained at 1 hour after the start of infusion and at Week 52, using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.

Twelve-lead ECGs should be measured with the subject in a semi-supine or supine position, having rested in this position for at least 5 minutes beforehand. It is preferred that the measurement be late with the subject rested, rather than on-time with the subject not sufficiently rested. If the subject is not sufficiently rested, this needs to be stated in the source documents. Electrocardiogram results will be recorded on the appropriate page of the eCRF.

8.2.5 Clinical Laboratory Analyses

Laboratory tests, including ALT and coagulation panel, will be closely monitored throughout the duration of the study. Investigators will receive flagged notification of any laboratory values that are outside of the normal range. Any abnormal laboratory test results (hematology, coagulation panel, clinical chemistry, urinalysis, or other laboratory parameters), including those that worsen from baseline or are felt to be clinically significant in the medical and scientific judgment of the investigator, are to be recorded as AEs or SAEs (Section 9.1.2).

However, any clinically significant safety assessments that are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition, are **not** to be reported as AEs or SAEs.

All laboratory tests with results that are significantly abnormal during participation in the study should be repeated until the values return to normal or baseline. If such values do not return to normal within a period judged reasonable by the investigator, the etiology should be identified and the sponsor notified.

Samples of blood, urine, saliva, and stool will be collected for study assessments. Any samples remaining at the end of the study may be stored for up to 15 years and analyzed to better understand the effect of DTX101 on hemophilia B or other related blood disorders. The choice to allow retention and future analysis will be optional.

8.2.6 Clinical Laboratory Parameters

The clinical laboratory parameters to be measured are listed in Table 8–1. Samples are to be collected at the time points specified in the Schedules of Events (Table 15–1 and Table 15–2 [clinical chemistry only]).

Table 8–1 Clinical Laboratory Parameters

Clinical chemistry:	Sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, creatine kinase, bilirubin (total and indirect), ALT, AST, ALP, gamma-glutamyl transferase, and lactate dehydrogenase
Hematology:	Complete blood count with differential
Urinalysis:	Specific gravity, pH, glucose, protein, blood (by dipstick), ketones (by dipstick), and microscopic examination (if blood or protein is found)
Other:	HBV surface antigen (HBsAg), HCV RNA, HIV
Coagulation panel:	PT/INR, fibrin degradation product (D-dimer), aPTT

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; PT/INR, prothrombin time/international normalized ratio.

Details for the preparation and shipment of samples will be provided by the central laboratory. Reference ranges for all parameters will be provided to the study site by the central laboratory.

If additional non-protocol specified laboratory assessments are performed at the site's local laboratory and result in a change in subject management or the results are considered clinically significant by the investigator (eg, SAE, AE, or dose modification), the results must be captured and sent to the sponsor along with other study data as defined in Section 9.1.2.

A laboratory parameter may be repeated if there is any concern about the values obtained. Laboratory values must be repeated if ≥2-fold the ULN or ≤0.5-fold the lower limit of normal.

8.2.6.1 Elevation of Liver Function Tests

In clinical studies with AAV-mediated gene transfer, a transient rise in liver transaminases and concurrent decline in transgene expression has been observed [Manno 2006; Nathwani 2011a; Nathwani 2014]. This has been hypothesized to be due to the activation of capsid-specific cytotoxic T lymphocytes (CTLs) and destruction of transduced liver cells [Mingozzi 2007]. However, in mice, T-cells activated against AAV capsid were not able to target and eliminate transduced hepatocytes [Wang 2007; Li C 2007; Li H 2007; Siders 2009] unless the wild-type AAV genome was present [Li C 2007].

The inability to reproduce the observed effects in animal models has made it difficult to assess the validity of the hypothesis or to develop strategies to overcome or minimize transaminase elevations. Despite a lack of clear resolution that activation of CTLs leads to a reduction in transgene expression from hepatocytes, appropriate precautions have been incorporated into this study. In addition to monitoring for capsid and FIX-specific T-cell activation, the use of oral steroids is allowed if a subject experiences elevations in liver transaminases.

If a subject experiences a >1.5-fold increase in LFTs following treatment with DTX101, the investigator should consider the initiation of steroid treatment as outlined in Section 8.2.6.2. The event should be recorded as an AE or SAE, as defined in Section 9.1.2, on the appropriate pages of the eCRF if it is felt to be clinically significant in the medical and scientific judgment of the investigator.

8.2.6.2 Treatment for Potential Immune Hepatitis

In the absence of a specific etiology, steroid treatment for potential vector-induced immune hepatitis will be allowed if there is a >1.5-fold increase from baseline in the subject's ALT that is considered related to treatment with DTX101 by the investigator. Within 24 hours of learning of the elevation, the site will repeat the test at their local laboratory and, if still elevated, will initiate steroid treatment.

The site is encouraged to consult with the medical monitor prior to initiating treatment. Based on available evidence, it is expected that vector-induced viral hepatitis will be self-limiting. Therefore, prednisone (or prednisolone) will be the sole medication used per the American Association for the Study of Liver Disease guidelines, with a slight modification:

- Week 1: prednisone 60 mg/day
- Week 2: prednisone 40 mg/day
- Week 3 and Week 4: prednisone 30 mg/day

Starting at Week 5, prednisone will be tapered by 5 mg/week until liver enzymes return to baseline levels. The use of prednisone will be recorded on the appropriate page of the eCRF.

8.3 Other Laboratory Parameters

8.3.1 Factor IX Inhibitor

Samples for FIX inhibitor will be collected at the time points specified in the Schedule of Events (Table 15–1) to monitor for an immune response to FIX. The Bethesda assay will be performed using a validated method. Details for the preparation and shipment of samples are included in the laboratory manual.

8.3.2 Neutralizing Antibodies to Adeno-Associated Virus rh10

Samples for neutralizing antibodies to AAVrh10 will be collected at the time points specified in the Schedule of Events (Table 15–1) to monitor for a humoral immune response to AAVrh10. The assay will be performed using a research method (enzyme-linked immunosorbent assay [ELISA]). Details for the preparation and shipment of samples are included in the laboratory manual.

8.3.3 Adeno-Associated Virus rh10 Binding Antibody IgG Assay

Samples for the AAVrh10 binding antibody IgG assay will be collected at the time points specified in the Schedule of Events (Table 15–1) to monitor for circulating anti-AAVrh10

antibodies. The assay will be performed using a research method (ELISA). Details for the preparation and shipment of samples are included in the laboratory manual.

8.3.4 Viral Shedding

Saliva, urine, and stool will be collected at the time points specified in the Schedules of Events (Table 15–1 and Table 15–2) to monitor for the presence of shed virus. The presence of DTX101 will be determined by quantitative polymerase chain reaction (qPCR). Subjects will be given an appropriate container to collect a stool sample at home. Saliva and urine samples will be collected at the site. In the event the assay is positive, subjects will continue to provide weekly samples until the results are negative on 3 separate occasions for each sample matrix. Details for the preparation and shipment of samples are included in the laboratory manual.

8.3.5 Cell-Mediated Immune Response

The presence of T-cells specific for AAVrh10 and FIX will be determined by an enzyme-linked immunospot (ELISPOT) assay. Samples will be collected at the time points specified in the Schedule of Events (Table 15–1). Details for the preparation and shipment of samples are included in the laboratory manual.

8.4 Genotyping

8.4.1 Factor IX Genotyping

At baseline, subjects will be asked to provide a single whole-blood sample for FIX genotyping. The objective of this research is to provide a background understanding to the etiology of the subject's hemophilia B and to investigate any relationship between genetic factors and the subject's response to DTX101. The choice to provide this sample will be optional. If analyzed, the results will be reported separately. If not analyzed, the sample may be stored for future biological testing for a period of ≤15 years.

8.4.2 Human Leukocyte Antigen Genotyping

At baseline, subjects will be asked to provide an additional whole blood sample for human leukocyte antigen genotyping. The sample will only be analyzed if the result from the ELISPOT assay for cell-mediated immune response to AAVrh10 is ≥200 spot-forming units/1 × 10^6 peripheral blood mononuclear cells (Section 8.3.5). The objective of this research is to investigate any relationship between genetic factors and the subject's immune response to DTX101. The choice to provide this sample will be optional. If analyzed, the results will be reported separately. If not analyzed, the sample may be stored for future biological testing for a period of ≤15 years.

9 Safety Monitoring and Reporting

9.1 Adverse Events and Serious Adverse Events

Adverse events will be assessed from the time the subject signs the ICF until their exit from the study.

At every study visit, subjects will be asked a standard nonleading question to elicit any medically related changes in their well-being.

In addition to subject observations, AEs identified from any study data (eg, laboratory values, physical examination findings, ECG changes) or identified from review of other documents (eg, subject diaries) that are relevant to subject safety will be documented on the AE page in the eCRF.

9.1.1 Definitions

9.1.1.1 Adverse Events

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study product or their clinical significance.

An AE is defined as any untoward medical occurrence in a subject enrolled into this study regardless of its causal relationship to study product. Subjects will be instructed to contact the investigator at any time after the subject signs the informed consent if any signs or symptoms develop.

A treatment-emergent adverse event (TEAE) is defined as any event not present before exposure to study product or any event already present that worsens in either intensity or frequency after exposure to study product.

9.1.1.2 Serious Adverse Events

An SAE is defined as any event that:

- Results in death
- Is immediately life threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity, or
- Results in a congenital anomaly/birth defect.

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

9.1.2 Safety Reporting

9.1.2.1 Adverse Events

All AEs reported or observed during the study will be recorded on the AE page in the eCRF. Information to be collected includes drug treatment, dose, event term, time of onset, investigator-specified assessment of severity and relationship to study product, time of resolution of the event, seriousness, any required treatment or evaluations, and outcome. Adverse events resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed to adequate resolution. The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all AEs.

Any medical condition that is present at the time that the subject is screened but does not deteriorate should not be reported as an AE. However, if the condition deteriorates at any time during the study, it should be recorded as an AE.

9.1.2.2 Serious Adverse Events

Any AE that meets SAE criteria (Section 9.1.1.2) must be reported to PPD Pharmacovigilance (PVG) immediately (ie, within 24 hours) after the time site personnel first learn about the event. The site should record all SAE information in the SAE eCRF and submit the report via the electronic data capture (EDC) system.

In the event of any fatal or life-threatening SAE, the investigator must immediately inform PPD PVG by telephone (Table 9–1) and report the SAE in the EDC system. If, for any reason, it is not possible to report the SAE in the EDC system (eg, the EDC system is unavailable), the site should record the SAE on the paper SAE Reporting Form and fax it to PPD PVG (Table 9–1). As soon as it is possible, any SAE reported via fax must be entered into the EDC system.

Table 9–1 PPD PVG Contact Information for SAE Reporting

Region	Contact Information
Rest of World	**Safety hotline**: [redacted]
	Safety fax[a]: [redacted]
North America	**Safety hotline**: 1[redacted]
	Safety fax[a]: [redacted]

Abbreviations: PVG, pharmacovigilance; SAE, serious adverse event.

a. This is the preferred fax number for all regions. The toll free fax number for North America can be provided upon request.

9.1.2.2.1 Expedited Reporting

The sponsor is responsible for reporting suspected, unexpected, serious adverse reactions (SUSARs) involving the study product(s) to all regulatory authorities and participating investigators in accordance with ICH guidelines and/or local regulatory requirements, as applicable. It is the responsibility of the investigator or designee to promptly notify the local institutional review board (IRB)/independent ethics committee (IEC)/institutional biosafety committee (IBC) of all SUSARs involving risk to human subjects. Serious adverse reactions

will be assessed for expectedness using reference safety information specified in the Investigator's Brochure, as applicable.

9.1.3 Assessment of Severity/Toxicity

The severity/toxicity, or intensity, of an AE refers to the extent to which an AE affects the subject's daily activities. The intensity of the AE will be rated as Grade 1, 2, 3, 4, or 5 using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03 [NCI CTCAE 2010]. The CTCAE provides descriptive terminology that can be used to standardize AE reporting. A severity/toxicity grade is provided for each AE term that is grouped by the highest level of MedDRA classification [NCI CTCAE 2010]. Specific symptoms and medical conditions have a clinical description for each level of severity/toxicity. In the event that an AE occurs during the study that is not captured by the CTCAE, the AE should be graded according to the following general guidelines:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[a].

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b].

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

Abbreviation: ADL, activities of daily living; AE, adverse event.

a. Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

b. Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Changes in the severity/toxicity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent do not require documentation of onset and duration of each episode.

9.1.4 Assessment of Causality

The investigator's assessment of an AE's relationship to study product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

The relationship or association of the study product in causing or contributing to the AE will be characterized using the following classification and criteria:

Unrelated: This relationship suggests that there is no association between the study product and the reported event.

Possible: This relationship suggests that treatment with the study product is causing or contributing to the adverse event (AE); ie, the event follows a reasonable temporal sequence from the time of the study product administration or follows a known response pattern to the study product, but could also be produced by other factors.

Probable: This relationship suggests that a reasonable temporal sequence of the event with the study product administration exists and, based upon the known pharmacological action of the study product, known or previously reported adverse reactions to the drug or class of drugs, or judgment based on the investigator's clinical experience, the association of the event with the study product seems likely. The event disappears or decreases on cessation or reduction of the dose of study product.

Definite: This relationship suggests that a definite causal relationship exists between the study product administration and the AE, and other conditions (concurrent illness, progression/expression of disease state, or concurrent medication reaction) do not appear to explain the event. The event reappears or worsens if the study product is re-administered.

9.1.5 Follow-Up of Subjects Reporting Adverse Events

All AEs must be reported in detail on the appropriate page in the eCRF and followed to satisfactory resolution, until the investigator deems the event to be chronic or not clinically significant, or until the subject is considered to be stable.

9.2 Procedures for Handling Special Situations

9.2.1 Pregnancy

The population under study is male; therefore, pregnancies will not be tracked.

9.2.2 Treatment Noncompliance

9.2.2.1 Overdose Management

An overdose is any dose of study product given to or taken by a subject that intentionally or unintentionally exceeds the dose, based on body weight (kg), described in Section 3.1. Overdoses without signs or symptoms do not need to be recorded as AEs; in case of any AEs associated with the overdose, these should be reported on the relevant AE or SAE section in the eCRF. The actual dose infused will be recorded on the appropriate page of the eCRF.

There is no treatment for overdose. All subjects will be closely monitored at the time of infusion for any adverse effects and supportive care will be administered at the discretion of the investigator, as needed, should an overdose be suspected.

9.2.2.2 Medication Errors

A medication error is defined as a mistake made in prescribing, dispensing, administration, or use of the study product. The treatment will be open-label and is to be administered by trained medical personnel at the site.

9.3 Data Safety Monitoring Committee

An independent DSMC will be responsible for monitoring safety data from the study. The DSMC will meet after all subjects in a dosing cohort have completed Week 6 of the study to review the safety data and provide their recommendation for progressing to the next dosing cohort.

The DSMC will meet on an ad hoc basis if an investigator reports an AE/SAE that meets any of the safety stopping criteria (Section 3.2.4). The DSMC may, at any time, recommend

modifying or stopping the study early due to safety concerns based on these periodic data reviews. The full scope of each review will be outline in the DSMC charter.

The DSMC will have 2 independent medical professionals and an independent biostatistician who are qualified to review the data and provide recommendations for progressing to the next dosing level. The DSMC charter details the members' roles and responsibilities as part of the DSMC as well as the process for each data review (ad hoc or scheduled).

10 Statistical and Analytical Plan

A statistical analysis plan (SAP) will be written and finalized prior to the start of the study and will provide a detailed description of the statistical methods and expand on the details provided in this protocol. Additional analyses may be added.

10.1 Dose-Finding Algorithm and Process

- Data sources and methodologies used to determine the parameter values of the prior distributions of the toxicity and efficacy models;
- Data elements and formats required in order to conduct modeling needed for producing dosing recommendation for the next dose;
- Validation process of the modeling results and format and data to be provided to the DSMC for dossing decisions.

10.2 Primary Endpoints

The primary endpoints are:

- The incidence of AEs, TEAEs, and SAEs (Section 9.1);
- The change from baseline in FIX activity at Week 6 (Section 8.1.1).

10.3 Secondary Endpoints

The secondary endpoints are:

- The annualized bleeding rate (ABR) (Section 8.1.2);
- The time course of FIX activity (Section 8.1.1);
- The annual and weekly use of FIX replacement therapy (Section 8.1.3);
- Development of FIX inhibitor (Section 8.3.1);
- Development of a cell-mediated immune response to FIX (Section 8.3.5);
- Quality-of-life assessments (Section 8.1.4).

10.4 Other Endpoints

Additional endpoints include:

- Vital sign measurements (Section 8.2.3);
- Electrocardiograms (Section 8.2.4);
- Clinical laboratory parameters (Section 8.2.6);
- Development of neutralizing antibodies to AAVrh10 (Section 8.3.2);
- Adeno-associated virus rh10 binding antibody IgG assay (Section 8.3.3);
- Viral shedding (Section 8.3.4).

10.5 Statistical Analysis Methodology

Dose-finding modeling will be conducted through specialized software that has been validated by PPD. SAS® software (SAS Institute, Inc, Cary, North Carolina, United States) Version 9.2 or later will be used for general data manipulation and statistical analyses. Continuous variables will be summarized using the mean, the standard deviation, median, minimum value, and maximum value. Categorical variables will be summarized using frequency counts and percentages. Data will be listed in data listings.

Details of the statistical analyses, methods, and data conventions are described in the SAP.

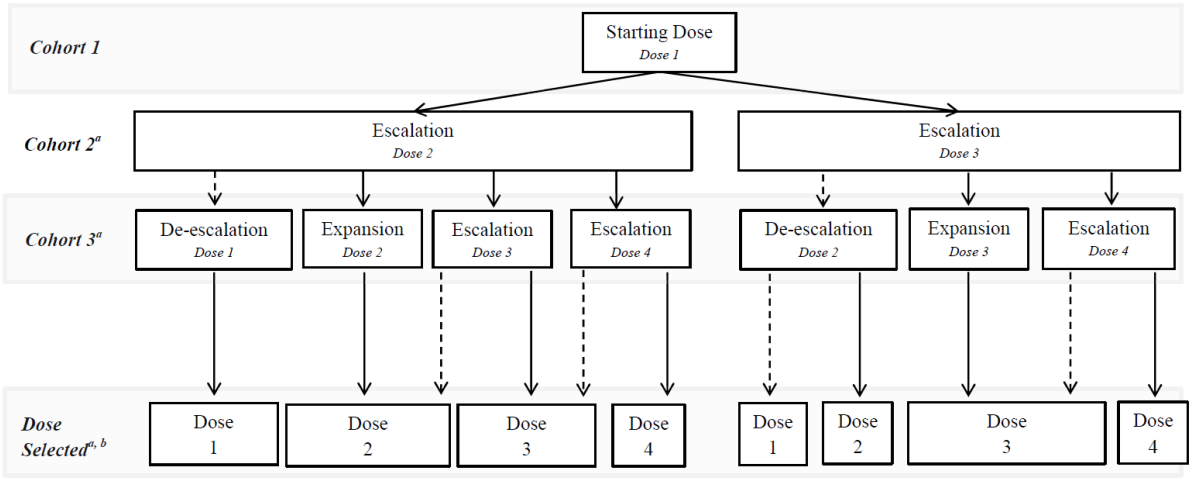
10.5.1 Determination of the Optimal Biological Dose

The continual reassessment method (CRM) chosen for the study is a bivariate approach [Braun 2002]. This method uses a 2-parameter logistic regression model to predict both the probability of experiencing a dose-limiting toxicity (DLT) and the probability of achieving the target peak FIX activity (≥20% of normal) within the first 6 weeks after DTX101 infusion. A noninformative prior [Braun 2002], in the form of a product of beta and exponential density functions, will be used. A dose will be considered the maximum tolerated dose (MTD) if the posterior probability of a DLT reaches 25%. For this study, a DLT is defined as any AE/serious AE (SAE) ≥Grade 3 that is considered to be related to study product by the investigator [NCI CTCAE 2010]. The MTD can also be the OBD if target FIX

activity is achieved. A dose will be considered the MED if the predicted probability of achieving the target peak FIX activity (≥20% of normal) reaches 90%. At each dose, a Euclidian distance between the posterior probabilities of safety and efficacy signals will be minimized to identify the dose for the next cohort.

Subjects will be dosed sequentially in cohorts of a minimum of 3 subjects each. Subjects in the first cohort (Cohort 1) will be assigned to Dose 1. Subjects will be assigned to subsequent cohorts depending on the safety and FIX activity of the preceding doses. The potential dosing scheme allows, at most, bypassing one dose level (eg, Dose 1 to Dose 3) during dose-finding. Similarly, the potential dosing scheme allows for de-escalation if the MTD is reached as well as cohort expansion to confirm a dose is the OBD (Figure 10–1). The protocol will also allow for adding an intermediate dose, relative to the candidate doses outlined in Section 3.1, if the CRM predicts it could be the OBD.

Figure 10–1 Overview of Potential Dosing Scheme to Determine the Optimal Biological Dose of DTX101



NOTE: The CRM predicts the probability of experiencing a DLT and the probability of the dose achieving the target FIX activity (≥20% of normal.)
Solid arrows = dose escalation or expansion; Dashed arrows = dose de-escalation.
a. Dose escalation (n=3), de-escalation (n=3), or expansion (n=3) is based on the safety and FIX activity at the preceding dose as modeled by the CRM.
b. An intermediate dose, relative to the planned candidate doses, could be added if the CRM predicts it could be the OBD.

Abbreviations: CRM, continual reassessment method; DLT, dose-limiting toxicity; FIX, factor IX; OBD, optimal biological dose.

Dose escalation will stop if at least one of the following conditions is met:

1) The MTD is reached;
2) The MED is found;
3) If a dose is sufficiently close to either the MTD or MED (within 5% credible interval);
4) If the posterior probability of a dose being either the MTD or the MED is over a threshold (10% and 20%, respectively);
5) If all of the dosing cohorts have been enrolled.

10.5.2 Efficacy Analysis

10.5.2.1 Annualized Bleeding Rate

The annualized number of bleeds per subject, per location, and by type of bleed will be calculated for all subjects through Week 52 (±7 days). Full details of the analysis will be provided in the SAP.

10.5.2.2 Factor IX Replacement Therapy

The annualized and average weekly use of FIX replacement therapy will be calculated for all subjects through Week 52 (±7 days). Full details of the analysis will be provided in the SAP.

10.5.3 Safety Analyses

All subjects who receive DTX101 will be included in the safety analysis.

10.5.3.1 Adverse Events

All statistical analyses of safety outcomes will be descriptive. The incidence of AEs and TEAEs will be summarized for each dosing cohort by severity and relationship to study product. Serious AEs will be presented for each dosing cohort by relationship to study product. Summary tables will present incidence estimates and individual event rates by system organ class as well as within each system organ class. Subjects experiencing an event

more than once with varying severity will be counted only once, in the maximum severity within each system organ class and preferred term. For incidence of relationship to study product, subjects will be counted only once, in the category of the strongest relationship to study product within each system organ class and preferred term.

10.5.3.2 Clinical Laboratory Assessment Results

For all laboratory assessments with continuous results, absolute values and changes from baseline will be summarized by visit and dosing cohort. For laboratory tests with categorical results, shifts from baseline will be summarized by visit and dosing cohort.

10.5.3.3 Vital Sign Measurements

Vital sign measurements (heart rate, blood pressure [systolic and diastolic], and respiratory rate) will be summarized over time in terms of absolute values and changes from baseline by visit and dosing cohort. Height and weight will be summarized.

10.5.3.4 Electrocardiogram Results

Electrocardiogram data will be summarized by visit and dosing cohort. Each ECG will be classified as “abnormal” or “normal,” and the relevance of the abnormality will be summarized as “clinically significant” or “not clinically significant.”

10.5.4 Pharmacodynamic Analyses

Factor IX activity will be summarized by time point and dosing cohort. Associations between FIX activity and dose will be undertaken using tabular summaries and appropriate statistical methods as outlined in the SAP.

10.5.5 Other Laboratory Results

Factor IX inhibitor, neutralizing antibodies (AAVrh10), AAVrh10 binding antibody IgG assay, viral shedding, and cell-mediated immune response (to AAVrh10) will be summarized by time point and dosing cohort. Associations between these parameters and dose will be

undertaken using tabular summaries and appropriate statistical methods as outlined in the SAP.

10.5.6 Quality-of-Life Assessments

Associations between quality of life and dose will be undertaken using tabular summaries and appropriate statistical methods as outlined in the SAP.

10.5.7 Other Analyses

Summary statistical analyses will be provided for demographics, medical history, hemophilia history, physical examination, and prior and concomitant medications. A summary of subject disposition will be prepared.

10.5.8 Interim Analysis

An interim analysis will be conducted when the last subject dosed completes Week 6 of the study. The purpose of this interim analysis is to determine FIX activity in all cohorts to determine the OBD of DTX101 and provide an initial safety and efficacy assessment of DTX101. Results and their dissemination will be at the sponsor's discretion. Any interim analyses conducted will not bias the conduct of the study. A detailed plan for the analysis of the safety and efficacy data will be presented in the SAP.

10.6 Data Quality Assurance

The sites will maintain source documentation and enter subject data into the eCRF as accurately as possible and will rapidly respond to any reported discrepancies. The eCRFs are accessed through Medidata Rave® (New York, New York, United States). This EDC system is validated and compliant with US Title 21 Code of Federal Regulations (CFR) Part A1. Each person involved with the study will have an individual user name and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records, as well as the time and date of any modifications. A quality review of the data will be performed by the site with additional reviews by the clinical monitor.

Each eCRF is presented as an electronic copy, allowing data entry by study site personnel, who can add and edit data, add new subjects, identify and resolve discrepancies, and view records. This system provides immediate direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner.

Paper copies of the eCRFs and other database reports may be printed and signed by the investigator. This system provides study site personnel, monitors, and reviewers with access to hardcopy audits, discrepancy reviews, and investigator comment information.

After all queries have been resolved, the SAP is approved and signed, and any summary/analysis populations are approved, the database will be locked. All summary and analysis of the data will be performed using SAS software Version 9.2 or later.

10.6.1 Data Management

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for the subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include diary cards, laboratory reports, ECG strips, and other materials.

The sponsor (or sponsor designee) will supply the eCRF. Study personnel must have documented training in the use of the EDC system before the system can be authorized.

All eCRF information is to be completed. If an item is not available or is not applicable, this fact should be indicated.

Investigative site personnel will enter subject data into the EDC system. The analysis data sets will be a combination of these data and data from other sources (eg, laboratory data). All entries and changes to the data in the eCRF will be recorded electronically with an audit trail specifying the date and time of entry or change and the name of the authorized person making the entry or change. The investigator will answer all queries issued, if applicable. Data queries and query correspondence will be included in the audit trail.

Clinical data management will be performed in accordance with applicable sponsor (or sponsor designee) standards and data cleaning procedures to ensure the integrity of the data (eg, removing errors and inconsistencies in the data). Adverse events and concomitant medication terms will be coded using MedDRA terminology.

After database lock, each study site will receive an electronic copy of their site-specific eCRF data as entered into the EDC system for the study, including full discrepancy and audit history. Additionally, an electronic copy of all of the study site's data from the study will be created and sent to the sponsor for storage. The sponsor (or sponsor designee) will maintain a duplicate electronic copy for their records. In all cases, subject initials will not be collected or transmitted to the sponsor.

11 Ethics

11.1 Institutional Review Board, Independent Ethics Committee, and Institutional Biosafety Committee

Federal regulations and the International Conference on Harmonisation (ICH) guidelines require that approval be obtained from an IRB/IEC/IBC before participation of human subjects in research studies. Before study onset, the protocol, ICF, advertisements to be used for the recruitment of study subjects, and any other written information regarding this study to be provided to the subject or the subject's legal guardian must be approved by an IRB/IEC/IBC. Documentation of all IRB/IEC/IBC approvals and of the IRB/IEC/IBC compliance with ICH harmonised tripartite guideline E6(R1): Good Clinical Practice (GCP) will be maintained by the site and will be available for review by the sponsor or its designee.

All IRB/IEC/IBC approvals should be signed by the IRB/IEC/IBC chair or designee and must identify the IRB/IEC/IBC name and address, the clinical protocol by title or protocol number or both, and the date approval or a favorable opinion was granted.

The investigator is responsible for providing written summaries of the progress and status of the study at intervals not exceeding 1 year or otherwise specified by the IRB/IEC/IBC. The investigator must promptly supply the sponsor or its designee, the IRB/IEC/IBC, and, where applicable, the institution, with written reports on any changes significantly affecting the conduct of the study or increasing the risk to subjects.

11.2 Ethical Conduct of the Study

The study will be performed in accordance with the ethical principles that have their origin in the Declaration of Helsinki, ICH GCP, and all applicable local laws and regulations.

11.3 Subject Information and Consent

A written ICF in compliance with regulatory authority regulations and 21 CFR §50 shall be obtained from each subject before entering the study or performing any unusual or nonroutine procedure that involves risk to the subject. An ICF template may be provided by the sponsor to investigative sites. If any institution-specific modifications to study-related

procedures are proposed or made by the site, the ICF should be reviewed by the sponsor or its designee or both before IRB/IEC/IBC submission. Once reviewed, the ICF will be submitted by the investigator to his or her IRB/IEC/IBC for review and approval before the start of the study. If the ICF is revised during the course of the study, all active participating subjects must sign the revised form.

Before recruitment and enrollment, each prospective subject or their legal guardian will be given a full explanation of the study and be given the opportunity to read the approved ICF. Once the investigator is assured that the subject or their legal guardian understands the implications of participating in the study, the subject or their legal guardian will be asked to give consent to participate in the study by signing the ICF.

The investigator shall retain the signed original ICF(s) and must provide a copy of the signed original form to the subject or their legal guardian.

12 Investigator's Obligations

The following administrative items are meant to guide the investigator in the conduct of the study but may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Any change will be reported to the IRB/IEC/IBC but will not require a protocol amendment.

12.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject (or the subject's legal guardian), except as necessary for monitoring and auditing by the sponsor, its designee, the FDA, other applicable regulatory agencies, or the IRB/IEC/IBC.

The investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor must be obtained for the disclosure of any said confidential information to other parties.

12.2 Financial Disclosure and Obligations

Investigators are required to provide financial disclosure information to allow the sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR §54. In addition, the investigator must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Neither the sponsor nor PPD is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, neither the sponsor nor PPD is financially responsible for further treatment of the subject's disease.

12.3 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ICH E6(R1) 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- IRB/IEC/IBC approvals;
- Original investigator-signed investigator agreement page of the protocol;
- Form FDA 1572 (or equivalent), fully executed, and all updates on a new fully executed Form FDA 1572 (or equivalent);
- Curriculum vitae for the investigator and each subinvestigator;
- Financial disclosure information to allow the sponsor to submit complete and accurate certification or disclosure statements required under 21 CFR §54. In addition, the investigators must provide to the sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study;
- IRB/IEC/IBC-approved ICF, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject or their legal guardian; and
- Laboratory certifications and normal ranges for any local laboratories used by the site, in accordance with 42 CFR §493.

12.4 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH E6(R1). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. Study information from this protocol will be posted on publicly available clinical study registers before enrollment of subjects begins.

12.5 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6(R1) and all applicable guidelines and regulations.

12.6 Adverse Events and Study Report Requirements

By participating in this study the investigator agrees to submit reports of SAEs according to the time line and method outlined in the protocol. In addition, the investigator agrees to submit annual reports to the study site IRB/IEC/IBC as appropriate.

12.7 Investigator's Final Report

Upon completion of the study, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC/IBC with a summary of the study's outcome and the sponsor and regulatory authority(ies) with any reports required.

12.8 Record Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the study product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

12.9 Publications

After completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the sponsor will be responsible for these activities and will work with the investigators to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues. The sponsor has final approval authority over all such issues.

13 Study Management

The administrative structure will include a DSMC (Section 9.3).

13.1 Monitoring

13.1.1 Monitoring of the Study

The clinical monitor, as a representative of the sponsor, has the obligation to closely follow the study. In doing so, the monitor will visit the investigator and study site at periodic intervals, in addition to maintaining necessary telephone and letter contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and personnel.

All aspects of the study will be carefully monitored, by the sponsor or its designee, for compliance with applicable government regulation with respect to current GCP and current standard operating procedures.

13.1.2 Inspection of Records

Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB/IEC/IBC review, and regulatory inspections by providing direct access to all study records. In the event of either an audit or inspection, the investigator agrees to allow the sponsor, representatives of the sponsor, or a regulatory agency (eg, FDA or other regulatory agency) access to all study records.

The investigator should promptly notify the clinical research associate (CRA) of any audits or inspections scheduled by any regulatory authorities and promptly forward copies of any reports received to the CRA. The CRA will then inform and forward any reports to the sponsor.

13.2 Management of Protocol Amendments and Deviations

13.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the subject, must be reviewed and approved by the sponsor or its designee. Amendments to the protocol must be submitted in writing to the investigator's IRB/IEC/IBC for approval before subjects can be enrolled into an amended protocol.

13.2.2 Protocol Deviations

A deviation from the protocol is a departure from the written procedures or processes. A significant deviation occurs when there is nonadherence to the protocol by the subject or investigator that results in a significant, additional risk to the subject. Significant deviations can include nonadherence to inclusion or exclusion criteria, enrollment of the subject without prior sponsor approval, or nonadherence to FDA regulations or ICH GCP guidelines, and will lead to the subject being withdrawn from the study (Section 7.1). Protocol waivers or exemptions are not permitted. Adherence to the study design requirements, including those specified in the Schedules of Events (Table 15–1 and Table 15–2), is essential for study conduct.

The investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study subjects without prior IRB/IEC/IBC approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB/IEC/IBC for review and approval, to the sponsor for agreement, and to the regulatory authorities, if required.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. Principal investigators will be notified in writing by the monitor of deviations. The IRB/IEC/IBC should be notified of all protocol deviations in a timely manner, as required.

13.3 Study Termination

Although the sponsor has every intention of completing the study, the sponsor reserves the right to discontinue the study at any time for clinical or administrative reasons.

The end of the study is defined as the date on which the last subject completes the last visit (includes follow-up visit).

13.4 Final Report

Whether the study is completed or prematurely terminated, the sponsor will ensure that the clinical study reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor will also ensure that the clinical study reports in marketing applications meet the standards of the ICH harmonised tripartite guideline E3: Structure and content of clinical study reports.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results.

Upon completion of the clinical study report, the sponsor will provide the investigator with a summary of the cumulative study results. The investigator is encouraged to share the cumulative summary results and will provide each subject with their individual data. The study results will be posted on publicly available clinical study registers, where required.

Declaration of Investigator

I have read and understood all sections of the protocol entitled "A Phase I/II Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) rh10-Mediated Gene Transfer of Human Factor IX in Adults With Moderate/Severe to Severe Hemophilia B."

I have read and agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the Final Protocol Version 04, dated 03 September 2015, the International Conference on Harmonisation harmonised tripartite guideline E6(R1): Good Clinical Practice and all applicable government regulations and inform all who assist me in the conduct of this study of their responsibilities and obligations.

Signature of Principal Investigator Date

Printed Name of Principal Investigator

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15 Appendices

15.1 Appendix: Schedules of Events

Table 15–1 Schedule of Events – Scheduled Clinic Visits

	Period	Screening	Dosing	Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see Table 15–2])							Unscheduled	End of Study/Early Withdrawal
	Week	Days −30 to −1	Day 0	2	4	6	8	10	12	16-48[a]		52
Procedure	Visit Window (Days)	-	-	±2	±2	±2	±2	±2	±2	±4	-	±7
Informed consent		X										
Demographics		X										
Medical history		X										
Hemophilia history		X										
Prior medication / therapies / procedures		X										
Review of eligibility criteria		X										
FIX genotyping		X										
HLA genotyping		X										
HBV, HCV, and HIV status		X										
FIX inhibitor (Bethesda assay)		X	X[b]			X	X			X[c]	X[d]	X

Procedure	Period	Screening	Dosing	Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see Table 15–2])							Unscheduled	End of Study/Early Withdrawal
	Week	Days −30 to −1	Day 0	2	4	6	8	10	12	16-48[a]		52
	Visit Window (Days)	-	-	±2	±2	±2	±2	±2	±2	±4	-	±7
Coagulation panel (PT/INR, D-dimer assay, aPTT)		X	X[b]	X	X	X	X	X	X	X	X[d]	X
AAVrh10 neutralizing antibody test (ELISA)		X	X[b]			X	X			X[c]	X[d]	X
Cell-mediated immune response to AAVrh10 and FIX (ELISPOT assay)			X[b]			X	X			X[c]	X[d]	X
AAVrh10 binding antibody IgG assay (ELISA)		X	X[b]			X	X			X[c]	X[d]	X
Saliva, urine, and stool for viral shedding (qPCR)[f]			X[e]	X	X	X	X	X	X	X	X[d]	X
FIX activity (aPTT clot-based assay)		X	X[b]	X	X	X	X	X	X	X	X[d]	X
Hematology / clinical chemistry[g]		X	X[h]	X	X	X	X	X	X	X	X[d]	X
Urinalysis		X		X	X	X	X		X		X[d]	X

Procedure	Period	Screening	Dosing	Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see Table 15–2])							Unscheduled	End of Study/Early Withdrawal
	Week	Days −30 to −1	Day 0	2	4	6	8	10	12	16-48[a]		52
	Visit Window (Days)	-	-	±2	±2	±2	±2	±2	±2	±4	-	±7
12-lead ECG		X	X[i]									X
Vital sign measurements (heart rate, blood pressure, respiratory rate)		X	X[j]	X	X	X	X	X	X	X	X	X
Height and weight[k]		X	X[b, l]									X[l]
Complete physical examination		X										X
Targeted physical examination[m]			X[b]			X			X	X[n]		
AE/SAE monitoring		X	X	X	X	X	X	X	X	X	X	X
Concomitant medications / therapies / procedures			X[b]	X	X	X	X	X	X	X	X	X
Diary distributed			X									
Diary reviewed				X	X	X	X	X	X	X	X	X
Diary returned												X

	Period	Screening	Dosing	Treatment Period: Week 2 through Week 52 (includes clinic or home visits [see Table 15–2])							Unscheduled	End of Study/Early Withdrawal
	Week	Days −30 to −1	Day 0	2	4	6	8	10	12	16-48[a]		52
Procedure	Visit Window (Days)	-	-	±2	±2	±2	±2	±2	±2	±4	-	±7
Record of spontaneous bleeding episodes		X	X[b]	X	X	X	X	X	X	X	X	X
Record recombinant FIX use		X	X[b]	X	X	X	X	X	X	X	X	X
Prophylactic recombinant FIX washout		X[o]				X[p, q]				X[r, s]		X[r, s]
EQ-5D-5L QoL questionnaire			X[b]							X[n]		X
Haem-A-QoL questionnaire			X[b]							X[n]		X
DTX101 infusion			X									

Abbreviations: AAVrh10, adeno-associated virus serotype rh10; AE, adverse event; aPPT, activated partial thromboplastin time; ECG, electrocardiogram; ELISA, enzyme-linked immunosorbent assay; ELISPOT, enzyme-linked immunospot; EQ-5D-5L, EuroQoL 5D 5 level; FIX, factor IX; Haem-A-QoL, Haemophilia-Specific Quality of Life; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IgG, immunoglobulin G; INR, international normalized ratio; PT, prothrombin time; QoL, quality of life; qPCR, quantitative polymerase chain reaction; SAE, serious adverse event.

a. Following the Week 12 visit, subjects will return to the study site once every 4 weeks starting at Week 16.

b. To be collected before DTX101 administration.

c. Samples to be obtained at Weeks 16, 32, 40, and 48.

d. A laboratory parameter may be repeated if there is any concern about the values obtained. Laboratory values must be repeated if ≥2-fold the upper limit of normal or ≤0.5-fold the lower limit of normal.
e. Saliva and urine samples for viral shedding to be collected before DTX101 administration. Subjects will be provided with an appropriate container to collect a stool sample at home prior to the visit.
f. Samples for viral shedding to be collected at Weeks 2, 4, 6, 8, 10, and 12 and on Days 8, 20, 36, 48, 64, and 76 (see Table 15–2) until negative on 3 consecutive occasions for each sample matrix.
g. Hematology to include: complete blood count and differential. Clinical chemistry to include: sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, creatine kinase, bilirubin (total and indirect), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, and lactate dehydrogenase.
h. Samples for clinical laboratory assessments to be collected before DTX101 administration and 0.5, 4, and 8 hours after the start of the infusion.
i. A 12-lead electrocardiogram to be performed in triplicate before the start of infusion. A single 12-lead electrocardiogram to be performed 1 hour after the start of the infusion. The ECGs should be measured with the subject in a semi-supine or supine position, having rested in this position for at least 5 minutes beforehand.
j. Vital signs to be measured at predose, at 5 minutes after the start of the infusion, and at 0.5, 1, 2, 4, 6, and 8 hours after the start of infusion (±5 minutes). The predose measurement of heart rate and blood pressure is to be performed in triplicate, with measurements taken 2 minutes apart. Vital signs will also be measured at 22 hours (±1 hour) after the start of infusion, prior to subject discharge. Vital signs should be measured with the subject in a semi-supine or supine position, having rested in this position for at least 5 minutes beforehand.
k. Height to be collected at screening only.
l. Only weight to be collected.
m. Targeted physical examination to include assessment of the skin and the respiratory, cardiovascular, and gastrointestinal systems.
n. To be recorded at Weeks 24, 36, and 48.
o. This is only applicable if a subject does not have a documented history of FIX activity and a baseline sample is needed to confirm severity of hemophilia B. For subjects taking long-acting recombinant FIX prophylactically, washout is to start at Day –28 and last at least 21 days (Day –7). For subjects taking traditional recombinant FIX prophylactically, washout is to start at Day –14 and last at least 7 days (Day –7). The baseline sample must be obtained and results available prior to dosing on Day 0.
p. For subjects who take long-acting recombinant FIX prophylactically, start washout at Week 3 for the Week 6 FIX measurement.
q. For subjects who take traditional recombinant FIX prophylactically, start washout at Week 5 for the Week 6 FIX measurement.
r. For subjects who continue to take long-acting recombinant FIX prophylactically, start washout at Week 21 for the Week 24 FIX measurement and again at Week 49 for the Week 52 FIX measurement.
s. For subjects who continue to take traditional recombinant FIX prophylactically, start washout at Week 23 for the Week 24 FIX measurement and again at Week 51 for the Week 52 FIX measurement.

Table 15–2 Schedule of Events – Clinic or Home Visits During the Treatment Period

		Treatment Period											
	Day	**4**	**8**	**20**	**24**	**32**	**36**	**48**	**52**	**60**	**64**	**76**	**80**
Procedure	**Visit Window (Days)**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**	**±1**
Clinical chemistry[a, b]		X	X	X	X	X	X	X	X	X	X	X	X
Saliva, urine, and stool for viral shedding (qPCR)[c, d]			X	X			X	X			X	X	

Abbreviations: qPCR, quantitative polymerase chain reaction.

a. Clinical chemistry to include: sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, glucose, calcium, phosphate, magnesium, albumin, total protein, creatine kinase, bilirubin (total and indirect), alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, and lactate dehydrogenase.

b. A laboratory parameter may be repeated if there is any concern about the values obtained. Laboratory values must be repeated if ≥2-fold the upper limit of normal or ≤0.5-fold the lower limit of normal.

c. Subjects will be provided with an appropriate container to collect a stool sample at home prior to the visit.

d. Samples for viral shedding to be collected at Weeks 2, 4, 6, 8, 10, and 12 (see Table Table 15–1) and on Days 8, 20, 36, 48, 64, and 76 until negative on 3 consecutive occasions for each sample matrix.